SOIL HEALTH RESPONSE TO CROPPING SYSTEMS

IN SEMI-ARID MONTANA

by

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ABSTRACT

Traditional cropping systems in the northern Great Plains (NGP) were dominated by cereal-fallow rotations until the 1970s, resulting in increased soil erosion, decreased soil organic matter (SOM) accumulation, and declines in soil biological activity. Recent shifts toward continuous and more diverse no-till crop production attempt to increase sustainability, diversify economic opportunities, and keep up with the growing food demand without converting more land into agriculture.

With a two-year study, I explored the effects of crop types in diverse, no-till, crop sequences on soil health in dryland and irrigated systems on one farm in semi-arid Montana, using biological indicators of potentially mineralizable nitrogen (PMN), soil enzyme activity (β-glucosaminidase, β-glucosidase, arylsulfatase, and acid and alkaline phosphatases), and permanganate oxidizable carbon (POxC), a measure of labile carbon. Crop sequences included four crop types – cereals, oilseeds, legumes, and root crops. Root crops, namely sugar beet, drove soil responses in PMN, evident by increased plant-available N in soils following sugar beet. Soil enzyme activity, an indicator of nutrient cycling capacity, was strongly correlated with SOM, but did not follow a pattern based on crop type. Labile carbon changed in soils between years but did not respond consistently to crops.

This research also explored the soil health gap by comparing soil health in cultivated systems to nearby grasslands. In a paired-site comparison on two farms in Montana, biological health indicators were 45% lower, on average, in cultivated soils compared to adjacent uncultivated soils. This difference was consistent with lower SOM averages, offering a simple assessment to quantify the maximum attainable soil health capacity within a specific agroecosystem. Soil acidification, a growing concern for producers across the NGP, contributed to 42% lower soil enzyme activity, based on four enzymes, compared to adjacent neutral pH cultivated soils. Enzyme activity was the only soil health parameter that was lower in acid soils compared to neutral pH soils, demonstrating the sensitivity of soil enzymes.

Overall, these results indicate that biological soil health indicators are sensitive to changes in crop production, changing yearly, and provide farmers with the opportunity to fine-tune their management practices to meet their soil health goals.

CHAPTER ONE

INTRODUCTION

Crop Production in the Northern Great Plains

Global demand for crop production and livestock systems is increasing in response to an ever growing population, estimated to reach nearly 10 billion people over the next few decades (Gu et al., 2021), with a projected need of 35 – 56% more food by 2050 (van Dijk et al., 2021). This puts stress onto our current agricultural systems to keep up with the growing food demand by either intensifying current cropping systems or through conversion of more intact grasslands into cultivation (Tilman et al., 2011), both of which have ramifications for soil, water, and air quality, as well as socioeconomic implications if not done sustainably. Even with intensification, cropping systems will be required to maintain a high level of production, sustain the environment, preserve natural resources, and support the livelihood of the world's farmers (Baulcombe et al., 2009).

Traditional farming practices in the semi-arid northern Great Plains (NGP) region of the United States, and in Montana, include small grain cereals in rotation with summer fallow (Aase & Pikul, 2000). Summer fallow, the practice of leaving a field plant-free in an interannual cycle with wheat or barley, aims to accumulate soil moisture and nutrients in semi-arid agricultural regions to maintain high yields of dominant cash crops. While inclusion of summer fallow in cereal rotations can produce greater yields than continuous cereal production (Smika, 1970), there are depreciable impacts to soil quality, including increased soil erosion (Sharratt et al.,

2018), decreased soil organic matter (SOM) accumulation (Bowman et al., 1999; Engel et al., 2017), and declines in soil biological activity (Acosta-Martínez et al., 2007).

There has been a shift in agricultural practices in the NGP over the past three decades away from summer fallow, intensive tillage, and continuous cereal production, toward more diverse and no-till cropping systems (Hansen et al., 2012). No-till crop production avoids soil loss through wind and water erosion via maintenance of crop residues on the soil surface, which also increases water storage through soil protection and reduced evaporative loss (Tanaka et al., 2010a). No-till has also been adopted in some irrigated cropping systems due to its ability to reduce soil erosion and build SOM (Halvorson et al., 2006; Lal, 2004). Further, increasing crop diversity in both dryland and irrigated crop production has been proposed as a way to increase the overall sustainability of cropping systems while providing increased economic opportunities for farmers (Archer et al., 2020; Miller et al., 2015). Inclusion of oilseed and pulse crops in rotations with small grains has increased in the NGP over recent decades, largely due to changes in crop markets and government policies, but also because of increased grower support through Extension Services and research that drive crop diversification (Hansen et al., 2012). Beyond the positive impacts of crop diversity for soil physical properties, namely water infiltration and erosion protection (Tamburini et al., 2020), there are many soil chemical and biological benefits.

Increasing crop diversity over spatial and temporal dimensions through crop rotations and intercropping is associated with improved soil health, pest control, and increased nutrient cycling (Duru et al., 2015). Various crop types have differing impacts to agricultural biodiversity and the potential for soils to provision ecosystem services (Moss et al., 2019). Pulse crops develop symbiotic relationships with soil microbes, leading to biological nitrogen (N) fixation supplying

plant available N to the soil mainly through nodules on roots, and also aboveground crop residue returns (Lal, 2017). Inclusion of pulse crops in rotations can reduce the need for synthetic N fertilizer (Zentner et al., 2002), contributing a potential N credit, dependent on the quantity of N fixation compared to the N removed through harvested seed and straw (Ennin et al., 2004). Oilseed crops are primarily utilized in rotations for economic reasons such as market diversity and higher crop prices (Johnston et al., 2002). Some oilseed crops produce substantial amounts of crop residues that provide increased soil cover after harvest (Babu et al., 2014). The high C:N residues of sunflower specifically can persist for long periods in more temperate climates, retaining the highest amount of residue cover over the first 100 days after harvest, at over 80% retention compared to wheat and pea, which retain roughly half of their initial cover (Ordóñez-Fernández et al., 2007). These oilseed residues have the ability to deliver nutrients to the soil slowly over their decomposition process (Babu et al., 2014). Cereal crops, the most common crop type grown in the NGP, is also associated with high quantities of high C:N residues (Blanco-Canqui & Lal, 2009), with C inputs acting as a driver of replenishing the soil organic carbon (SOC) pool and building SOM (Shrestha et al., 2013). Ultimately, diversifying the plant community can influence the diversity of soil organisms in the soil environment and can perpetuate biological synergies within cropping systems, affecting soil health in the long term (Tanaka et al., 2010b).

Soil Health in Cropping Systems

Soil health is an ambiguous term that has little meaning without context. Often, soil health is described as the degree to which soil does what we want it to do, emphasizing the importance of understanding its beneficial use and desired state to provide that context. Soil

quality is defined as a soil's fitness for use (Larson & Pierce, 1991) and the capacity for a soil to function (Karlen et al., 1997). This intricately links soil quality with function for a specific land use, which in agricultural landscapes relates directly to a soil's ability to sustain plant and animal productivity. Soil health relates to soil as a finite and dynamic living resource (Lal, 2016), defined as the "capacity of soil to function as a vital living system to sustain biological productivity, maintain environmental quality and promote plant, animal, and human health" (Doran & Zeiss, 2000). This focus on soil as a living system integrates function, ability to sustain productivity, and the importance of maintaining soil health for future generations.

Soil health cannot be measured directly, so parameters and traits related to a soil's physical, chemical, and biological properties are utilized as a proxy to determine how well a soil functions for its intended use (NRCS, 2015). But to make inferences about a soil's health, specifically in agricultural or other highly managed systems, it is most useful to measure parameters indicative of dynamic properties, or those most responsive to change. Soil biological indicators are critical for assessing soil health because of their sensitivity to land management practices and climatic variations; they often provide an indication of a trajectory change in quality long before other soil parameters respond (Doran & Zeiss, 2000).

The living component of soil represents a relatively small fraction (<0.05%); however, soils have the greatest microbial diversity of any ecosystem, with an estimated one billion bacteria, of up to one million different species, in a single gram of soil (Lehman et al., 2015a). Bacteria and other prokaryotes, archaea, only account for one fraction of soil biota, and drive soil-atmosphere gas exchange, like nitrification and N fixation, and the rapid turnover of organic and mineral compounds (Aslani et al., 2022). Biota is also composed soil fungi, protists, and

animals which contribute to plant nutrition, decomposition, and mediation of diseases (Aslani et al., 2022). This incredible diversity contributes to the multifunctionality and processes carried out by soil biota (Delgado-Baquerizo et al., 2016). The complex interactions between soil biota drive a soil's capacity to provision ecosystem services, support water and nutrient cycling, increase decomposition rates, and enhance biodiversity (Norris et al., 2020).

Early assessment of biological parameters included bulk activity measurements of respiration, microbial biomass, and presence of keystone species such as arbuscular mycorrhizal fungi (Lehman et al., 2015b). Over the years, measurements such as N mineralization potential, microbial diversity, soil fauna functional guilds, and extracellular enzymatic activity were added to soil quality assessments (Creamer et al., 2022). Based on the simplistic definition of soil health as "the capacity of a soil to function", biological indicators prove necessary for understanding and assessing soil health.

Biological Soil Health Indicators

There is incredible diversity among soil health parameters that allow for inferences of soil multifunctionality. This thesis will focus on biological soil health indicators that link directly to functional outcomes related to nutrient cycling (Creamer et al., 2022). Specifically, this research will explore soil extracellular enzyme activity, potentially mineralizable nitrogen, and permanganate oxidizable carbon in cropping systems.

Extracellular enzymes are released into soils primarily through the metabolic processes of soil fungi and bacteria but can also be released from dead organisms during the decomposition process (Dick et al., 1996). Upon secretion into the soil matrix from living bacteria and fungi, some enzymes become stable and can persist in soil for years while catalyzing biogeochemical

reactions and organic matter decomposition (Nannipieri et al., 2018). Natural selection will tend to promote enzyme production strategies that minimize C and nutrient costs to the cell while maximizing associated benefits (Allison et al., 2011). Their production is a foraging strategy by the cell, evolved to align nutrient and energy supplies with demand (Burns et al., 2013). Extracellular enzymes provide an array of multifunctionality in soil, increasing rates of nutrient cycling, assimilating plant nutrients, and leading the decomposition process, making them good candidates for a general indicator of microbial activity and soil health (Dick, 1994).

Each enzyme catalyzes a specific reaction, and the presence of those enzymes is influenced by not only the soil biota but also the substrate present (Dick, 1994). Most soil enzymes that are frequent foci of soil health assessments are those indicative of nutrient cycling potential. Phosphatases catalyze the hydrolysis of ester-phosphate bonds, releasing phosphate (P), which can then be used by plants and soil biota (Nannipieri et al., 2011). Carbon cycling enzyme β-glucosidase catalyzes the hydrolysis of β-D-glucopyranosides in the final rate-limiting step of cellulose decomposition, providing simple sugars to the soil microbial population (Stott et al., 2010). Indicator of N cycling capacity, β-glucosaminidase catalyzes the hydrolysis of N-acetyl-β-D-glucosamine, resulting in chitin degradation and amino sugars, a major source of mineralizable N in soils (Ekenler & Tabatabai, 2004). Arylsulfatase catalyzes the hydrolysis of ester sulfates, leading to available soil sulfur (S) (Tabatabai & Bremner, 1970). Rather than measuring the fluxes or pools of P, C, N, and S directly, assays of these enzymes can quantify the potential for reactions in soil that are linked to the cycling of these specific elements (Norris et al., 2020).

Cycling of N is a key function of soil health. Potentially mineralizable nitrogen (PMN) is a soil health indicator strongly linked to the N fraction of soil that is susceptible to mineralization, which provides a major source of N for crop uptake (Mahal et al., 2018). The organic N that is exuded by plant roots, decomposing plant materials, and microbes can be converted into inorganic, plant-available N forms by microbes, and the rates of these transformations determine potential N availability from microbial activity within the soil (Liptzin et al., 2023). The assessment of PMN measures these transformations to plant available N under specific temperature and moisture conditions, approximating the amount of N that would likely be mineralized across a growing season (Keeney, 1982). This N source can impact N fertilizer needs in cropping systems and is very sensitive to changes in land management, increasing with crop diversity, limited tillage, and incorporation of pulse crops (Liebig et al., 2006; Mahal et al., 2018).

Soil labile C is the SOC pool directly available for microbial activity, and is often considered to be a primary energy source for microorganisms (Haynes, 2005). Organic matter additions through crop residue and reduced tillage directly affect this labile organic C pool (Cooper et al., 2016). Permanganate oxidizable C (POxC), often referred to as active C, is comprised of the C derived from SOM and microbial biomass, and also contains compounds like lignin and complex polysaccharides (Bongiorno et al., 2019). The oxidation process of POxC mimics microbial decomposition of organic matter (Bongiorno et al., 2019), but is also affected by the chemistry of mineral fractions of soil (Woodings & Margenot, 2023). However, POxC is the labile C fraction most strongly correlated with SOC and other soil chemical, physical, and biological indicators of nutrient cycling, SOM, and microbial biodiversity and activity

(Bongiorno et al., 2019). Further, POxC is very sensitive to changes in land use and management practices, responding to reduced tillage, cover crop treatments, and high residue cropping systems within a single growing season, and is much more sensitive than SOC, which can take years to respond meaningfully to change (Wang et al., 2017). Compared to SOC, POxC estimates a C pool much more closely associated with soil biological function (Weil et al., 2003).

Research Objectives

The goal of my research was to provide a knowledge base on how soil biological health indicators respond to changes in management practices over short time scales. Specifically, I hope to contribute to the understanding of how soil extracellular enzymes, PMN, and POxC respond to crop types within diverse dryland and irrigated cropping systems in semi-arid Montana. Further, I aim to measure differences in soil health measures between soils under long-term crop production and adjacent uncultivated soils within a particular agroecosystem in an effort to set realistic soil health goals relative to a specific soil.

In Chapter Two, I investigate the effects of various crop types on soil biological activity in very diverse irrigated and dryland cropping systems on one farm in semi-arid southeastern Montana. I address how soil biological parameters of five soil enzyme activities, PMN, and POxC respond to the variation in quantity and quality of crop residue associated with different crop types after two growing seasons. For this assessment, I sampled four fields (two dryland and two irrigated) over two years, after the fields were cropped with a different crop type (cereals, oilseeds, legumes, and root crops). This assessment allows for a comparison of how the same soil responds to two different crop types in two years with respect to nutrient cycling capacity, nutrient availability, and labile C dynamics.

In Chapter Three, I focus on the soil health gap, a concept introduced by Maharjan et al. (2020), between soils under long-term crop production and adjacent uncultivated grassland soils. I sampled multiple agricultural fields with differing management practices, paired with nearby uncultivated soils, to quantify the overall gap in soil biological indicators of soil health on two farms in semi-arid Montana. This assessment allows for inference of the impact of land use changes and cultivation on soil biological activity compared to uncultivated grassland soils without the influence of crop production.

Acidification is a factor of intensive crop production, over fertilization of N (specifically in ammoniacal form), and poor N use efficiency (Yadav et al., 2020). In Montana and across the NGP, acidification is a growing issue of serious concern for producers, contributing to declines in productivity and crop yields (Reeves & Liebig, 2016). In Chapter 3, I explore the impacts on soil health from acidification. I examined a single field with areas of acidification paired with adjacent productive soils with a neutral pH to assess the impact of soil acidity on soil biological activity and to understand if acidification results in an additional decline in soil health.

With the research conducted in this thesis, I aim to increase the understanding of how indicators of biological soil health respond to crop types in diverse sequences under different cropping systems. Further, I set out to provide reasonable and relative soil health targets for farmers in semi-arid Montana.

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CHAPTER TWO

CROP TYPE EFFECTS ON SOIL BIOLOGICAL HEALTH IN SEMI-ARID MONTANA

Abstract

In semi-arid agricultural systems typical of the northern Great Plains, crop residue is the most readily available and sustainable source of organic input for building soil health. Different crop types used in diverse cropping systems contribute varying quantity and quality of crop residues to the soil. We conducted on-farm research in Forsyth, Montana, to compare the effects of different crop types and their associated residues that persisted overwinter on soil biological response, following cereals (winter wheat, spring wheat, and millet), legumes (soybean, yellow pea, and faba bean), oilseeds (sunflower), and root crops (sugar beets) in both dryland and irrigated fields over two years. Specifically, we measured biological indicators of nutrient cycling capacity because of their sensitivity to changes in land management practices, namely potentially mineralizable nitrogen (PMN), soil enzyme activity, and permanganate oxidizable carbon (POxC). PMN, a proxy of the N that may become biologically released during the growing season, was higher in soils following a root crop than oilseeds or cereals ($p < 0.05$), but similar following an intercropped legume-cereal or irrigated legumes alone ($p > 0.1$). The geometric mean of extracellular enzyme activity, a measure of nutrient cycling capacity, was positively correlated with soil organic matter over both study years ($r = 0.61$, $p < 0.001$). Soil POxC, an indicator of labile C, was higher following an oilseed than cereals or cereal/legume intercrop in the first study year ($p < 0.05$), but higher following cereals and irrigated legumes

than root crops or dryland legumes in the second year ($p < 0.05$). Overwintered crop residue was not a robust indicator of soil biological response, based on a lack of consistent correlations between quantity of crop residue and soil biological activity that persisted over both study years.

1. Introduction

In the semi-arid agricultural systems typical of the northern Great Plains (NGP), greater crop residue returns is a sustainable way of building soil health (Fu et al., 2021; Turmel et al., 2015) and notably soil organic carbon (SOC) (Engel et al., 2017). While production of crop residues has increased in recent years due to widespread no-till management and increased agricultural productivity (Suryatapa, 2023), demand and removal of residues has also increased for animal feed, biofuel production, and optimizing short-term profits (Blanco-Canqui & Lal, 2009; Muth & Bryden, 2013; Shinde et al., 2022). However, this could have sustainability impacts, as there are numerous soil health and economic benefits of retaining crop residue cover after harvest (Wilhelm et al., 2007). While the primary function of crop residues is to conserve soil and water (Blanco & Lal, 2023), these crop remnants can also be a vital source of nutrient return to the soil (Fu et al., 2021). Throughout the plant growth cycle, soil nutrients are used to meet the demands of a crop's basic cellular processes and for building plant tissues, and a fraction of these nutrients then leave the farm in harvested products. Between 0.05 – 0.22 Mg ha^{-1} of nitrogen (N) is removed through harvest of N intensive annual crops such as soybean and corn (Masters et al., 2016). If crop residue is retained, up to 75-80% of remaining residue can be mineralized into plant-available nutrients (Grzyb et al., 2020), with a portion of these nutrient available for the next year's crop (Rangarajan, 2009). Residues also provide physical benefits to soil, such as improved aggregation, increased water holding capacity, and erosion protection

(Turmel et al., 2015). These organic materials are necessary to maintain or build the SOC and soil organic matter (SOM) pools of agricultural soils (Muth et al., 2013; Turmel et al., 2015; Wilhelm et al., 2007). Further, crop residue and SOC is a primary food and energy source for the soil biotic community, driving microbial activity and soil biological traits (Bandick & Dick, 1999; Blanco & Lal, 2023; Khatoon et al., 2017). The numerous benefits to chemical, physical, and biological properties of agricultural soils demonstrate the importance of retaining crop residues to increase both soil health and the overall sustainability of agricultural systems in the NGP.

The semi-arid NGP of North America is known for its fertile soils and vast production of cereal crops, namely spring wheat, winter wheat, and barley (Krupinsky et al., 2006). However, there has been a shift away from traditional spring wheat-fallow rotations, and cereal monoculture systems, toward more diverse cropping systems to increase environmental and economic sustainability (St. Luce et al., 2020; Zentner et al., 2002). Inclusion of crop diversity, such as pulse crops and oilseeds, increases cropping system efficiency, sustains high grain and protein yields, aids in combatting diseases, can enhance soil fertility, and increases economic opportunities (Krupinsky et al., 2006; St. Luce et al., 2020; Zentner et al., 2002). However, there may be varying impacts on soil health, nutrient availability, and microbial activity associated with diverse crop rotations, specifically because of variation in the quantity and quality of crop residues (Chen et al., 2014).

Dominant annual crop types, depending on area, used in diverse agricultural systems of Montana are cereals, pulse crops, oil seeds, and lastly sugar beets (USDA-NASS, 2023). Cereal crops are high-residue crops and rich in carbon (C) relative to N, resulting in high C:N, with C

inputs acting as a driver of replenishing the SOC pool and building of SOM (Shrestha et al., 2013). Wheat is one of the largest residue contributors, emphasizing the importance of residue management in semi-arid cereal crop rotations (Blanco-Canqui & Lal, 2009). Pulse crops have the capacity to fix atmospheric N into plant available forms, which is vital for maintaining nutrient levels for N-demanding crops, making legumes a critical part of crop rotations for soil fertility (Rangarajan, 2009) and profitability (Burgess et al., 2012; Miller et al., 2015). This N benefit can contribute a legume N credit, often resulting in decreased N fertilizer need for succeeding crops (Burgess et al., 2012; Krupinsky et al., 2006). Oilseed crop residues are also nutrient-rich, with high C:N, and have been associated with less residue than wheat (Babu et al., 2014). Sugar beets, a root crop, often contribute less residues, especially belowground, after harvest, meaning they contribute less organic matter to soil, especially when compared to cereal crops (Götze et al., 2016). However, their residues have a narrow C:N (i.e. high quality), allowing for rapid decomposition by microbes and increased nutrient availability for plants (Nicolardot et al., 2001), often supplying appreciable plant available N to the soil (Moraghan & Smith, 1996). The quality and quantity of crop residues, combined with abiotic conditions, principally soil moisture and temperature, determine decomposition rates and the ability for residues to return nutrients to the soil.

The quantity of crop biomass, and its associated residues, have been correlated with increases in soil biological activity at the start of the following growing season in semi-arid agricultural systems (Housman et al., 2021). Soil biological parameters indicative of nutrient cycling capacity, N availability, and SOM dynamics can help distinguish how various crop types and their residues affect soil health. Therefore, we conducted on-farm research to measure

biological soil health indicators in the spring, after soils were cropped with different crop types, and when crops are beginning to grow and are likely to be influenced by both nutrient availability and biological activity. We investigated the effects of various crop types, in differing crop sequences across irrigated and dryland crop productions, on soil biological activity following cereals, legumes, an oil seed, and a root crop. These crop types were selected because of their variations in residue inputs to soil, both in quantity and quality, and because they are utilized in very diverse irrigated and dryland rotations in the semi-arid NGP.

The soil biological traits analyses are all drivers of soil function. Potentially mineralizable nitrogen (PMN) is the N concentration that is likely to mineralize into plant available forms of N under specific temperature and moisture conditions throughout the growing season (Keeney, 1982). PMN has been shown to increase in cropping systems with diverse rotations and limited tillage, and with incorporation of pulse crops (Liebig et al., 2006). Soil extracellular enzymes are released from bacteria and fungi and can persist in the soil matrix for weeks to years, catalyzing biogeochemical reactions and organic matter decomposition (Nannipieri et al., 2018). Changes to soil enzyme activity have been linked to management activities, such as crop rotations, tillage practices, and fertilizer management (Acosta-Martinez et al., 2011). Soil enzyme activity also responds more quickly to these management changes than other soil health measures such as SOM, which can take years to respond to changing conditions (Lehman et al., 2015).

Permanganate oxidizable carbon (POxC) represents the active and biologically available organic matter in soil, providing an important indicator of net N mineralization and agronomic performance (Weil et al., 2003). This indicator is the labile C fraction most strongly correlated

with nutrient cycling capacity, microbial activity, and the qualitatively variable SOM fraction, ultimately reflecting the potential for microbial decomposition of SOM (Bongiorno et al., 2019). Soil POxC is also representative of the chemically defined and mineral fraction of soil C dynamics (Woodings & Margenot, 2023). Concentrations of POxC are very responsive to changes in fertilizer applications and organic amendments (Wang et al., 2017). Chemical soil quality parameters of total C, total N, and C:N are indicative of the rate at which microbes can decompose organic materials and make nutrients plant-available, controlling both mineralization and immobilization potential (Blanco et al., 2023). These soil health traits all provide indication of soil function and nutrient cycling capacity in agricultural soils.

Because of the variation in the quantity and quality of crop residues from different crop types, we expected that soil biological activity would differ between crop types. Specifically, with variation in residue quality (i.e., C:N) we hypothesized that crop types would affect PMN differently. We expected that soils with lower C:N crop residues, namely root crops and legumes, would result in more plant available N because of the ability of these residues to decompose and mineralize quickly. Likewise, crop residues with high C:N, like cereals and oilseeds, would decrease PMN. We also expected that crop types would impact soil extracellular enzymes, because of the variation in organic inputs and ability to build SOM from various crop types. SOM provides a food source for soil biota and increases microbial activity, in addition to stabilizing and protecting soil enzymes, providing greater nutrient cycling capacity in high SOM soils (Burns et al., 2013). Therefore, we hypothesized that crop types associated with SOM accrual, i.e. high residue crops like cereals and oilseeds, would be associated with higher enzyme activity.

Our second objective aims to assess how crop residue present on the soil surface after winter, at the beginning of the growing season, relates to soil biological activity. Because crop residue provides a consistent source of organic C to the soil, we expected that biomass of crop residue that overwinters would be an indication of labile C, POxC, when compared to soils with less crop residue. A greater quantity of crop residue is associated with increases in SOM (Engel et al., 2017), which directly relates to C storage and nutrient availability in soil (Turmel et al., 2015), and possibly linking with POxC with SOM. With this study design, we can compare the effects of different crop types, with variations in the quantity and quality of crop residue, on biological soil health indicators within diverse irrigated and dryland systems in the NGP.

2. Methods

2.1 Site Description

This study was conducted on a 2,307 ha, no-till, irrigated and dryland farm located west of Forsyth, Montana (46°15’54.33” N, 106°52’09.77” W), along the Yellowstone River. This area of east central Montana receives an average of 374 mm of precipitation annually, with an average temperature of 8.2 °C (Western Regional Climate Center, 2010a) reflecting a cold semi-arid climate type. The soil is a frigid, Aridic Ustifluvent loam (Soil Survey Staff 2022). Soil characteristics differed among fields (Table 2.1).

2.2 Agronomic Practices

This farm includes four primary crop types in rotations, including cereals, legumes, oilseeds, and root crops. The crops assessed in this study were winter and spring wheat (*Triticum aestivum L.*), foxtail millet (*Panicum italicum L.*), yellow pea (*Pisum sativum L.*), faba beans

(*Vicia faba L.*), soybeans (*Glycine max L.*), sunflowers (*Helianthus annuus L*), and sugar beets (*Beta vulgaris L.*) sequenced on four fields. A stripper header was used to harvest all cereal crops, resulting in maximum crop residue left on the fields after harvest. Legume and oilseed crops were harvested no-till with more traditional headers at the maximum stubble height while retaining high yields. Although the harvest of root crops involves some soil disturbance resulting in a thin layer of soil covering shredded crop residues, they were also managed no-till, providing a minimum soil disturbance and no soil mixing.

2.2 Experimental Design

The study consisted of four fields, two flood irrigated (i) and two dryland (d), at different points in their respective crop sequences. The fields were sampled after growing different crop types near the beginning of the plant growing season in 2022 and 2023, referred to as Year 1 and Year 2, to assess impacts to biological activity following various diverse crop types. Yield data from the four fields from both study years are summarized in Table 2.2. The first irrigated field (1) was cropped in a cereal-legume (C/L) intercrop of millet-faba bean in Year 1, followed by a root crop (R) of sugar beet in Year 2; this field is referred to as 1 C/L-R (i). The faba bean crop in this field did not flower or produce grain and volunteer millet also emerged in Year 1, resulting in a failed legume crop but successful cereal harvest. The second irrigated field (2) was cropped in sugar beet in Year 1 followed by a legume, soybean, in Year 2, and is referred to as 2 R-L (i). The lowland dryland field (3), was cropped in an oilseed (O) of sunflower in Year 1 followed by a legume of yellow pea in Year 2, referred to as 3 O-L (d). The upland dryland field (4) was cropped with a stacked cereal sequence of winter wheat in Year 1 followed by spring wheat in Year 2, referred to as 4 C-C (d).

The stacked cereal sequence of field 4 C-C is part of a longer rotation of winter wheat-spring wheat-lentil-yellow pea and has been cropped under this rotation for the last 30 years. Field 3 O-L, the other dryland field, is in the lowlands in proximity to the river and slightly downhill of other irrigated lands. These diverse crop types provided a range of physical and chemical traits of residue inputs to the soil. The irrigated and dryland fields selected for this study are representative of more diverse and unique dryland and irrigated cropping systems of southeastern Montana, where cereals in rotation with pulse crops and occasional oilseeds dominates the upland dryland crop production and a large variety of pulses, oilseeds, root crops, with an occasional cereal comprises the lowland soils that are primarily irrigated with some dryland production. This variety of rotation provided a unique opportunity to assess the impacts of a wide range of crops and crop types on soil biological activity in a semi-arid cropping system, under both dryland and irrigated conditions.

2.3 Sample Processing

Soil sampling and surface residue collection was performed on May 16, 2022, and May 1, 2023. Root biomass samples were also collected at the time of soil sampling in the first year of the study. Each field was divided into six sections in effort to capture within field variation, and seven soil cores (2.5cm dia x 15cm depth) were composited within each section, for a total of six composited soil samples per field. The soil corer was sterilized with 97% ethanol between samples. Field-moist soils were passed through a 2 mm sieve and stored at 4 °C for a maximum of 30 days prior to analyses. Soil textures were classified via Particle Size Analysis Hydrometer method (Gavlak et al., 2003). Soil pH was assessed with a 1:1 soil to water paste and measured with an Orion ROSS Ultra pH/ATC triode (USDA-NRCS, 2004).

Root cores (10 cm dia x 20.5 cm depth) were randomly located in each section within a field. Root cores were stored at 4 °C for a maximum of 60 days before dispersion in a 5% sodium hexametaphosphate solution, followed by a thorough rinse with water to remove all soil particles prior to assessment for structure and total root biomass. The surface residue associated with each root core (10 cm dia), including all residue on the soil surface and standing stalks, was collected at the time of sampling, oven dried at 50 °C for 72 h prior to assessment for dry weight of residue inputs. Crop residue amounts were then averaged across the field and extrapolated to Mg ha^{-1}.

2.4 Potentially Mineralizable Nitrogen

PMN was assessed using a 14-day anaerobic lab incubation at 30 °C (Keeney, 1982). Six flasks were filled with 5 g of field-moist soil and three of the flasks were immediately extracted in a 1M potassium chloride (KCl) solution and mechanically shaken for 30 minutes at 310 rpm. These soils were analyzed for ammonium (NH_4^+) at day zero. The headspace of the other three flasks were purged with N_2 gas for 5 seconds to create anoxic conditions, then incubated in darkness for 14 days at 30 °C. These soils were then extracted in a 1M KCl solution and shaken for 30 minutes at 310 rpm and assessed for NH_4^+ at day 14. All samples were analyzed on a Lachat autoanalyzer (Lachat Instruments, Loveland, CO) in Montana State University's Environmental Analytical Laboratory. PMN was calculated from the difference in pre- and post-incubation NH_4^+ concentrations and averaged across lab triplicates.

2.5 Soil Enzymes

Soil extracellular enzyme activity was analyzed following the procedure outlined by Dick et al. (1996) and Parham & Deng (2000). In duplicates, 1g of field-moist soil was incubated with

the p-nitrophenol enzyme-specific substrate at 37 °C for 1 h, then filtered and analyzed spectrophotometrically. Controls, in duplicates, received the enzyme-specific substrate immediately before filtration. Five enzymes were assessed, including β-1,4-N-acetyl glucosaminidase (EC 3.2.1.30, involved in N cycling), β-1,4-glucosidase (EC 3.2.1.21, involved in C cycling), arylsulfatase (EC 3.1.6.1, involved in sulfur cycling), and acid and alkaline phosphatases (EC 3.1.3.1/2, involved in phosphorus cycling). The geometric mean was calculated by taking the fifth root of the product of all enzyme activity, representing a measure of total soil extracellular enzyme activity that accounts for skewed data (García-Ruiz et al., 2008).

2.6 Permanganate Oxidizable Carbon

Soil POxC was analyzed in duplicates with 2.5 g of air-dried and pulverized soil, ground for 12 h to a fine consistency of <0.1 mm with a jar mill. The soils were combined with 18 mL of deionized water and 2 mL of 0.2 M potassium permanganate ($KMnO_4$) in 1 M calcium chloride ($CaCl_2$). The samples were shaken at 120 rpm for 2 min, then allowed to settle in the dark for 10 minutes at room temperature. An aliquot of 0.5 mL of the supernatant was dispensed into 49.5 mL of deionized water. The extraction produced a colorimetric response that was measured spectrophotometrically, at 550 nm, following methods developed by Weil et al. (2003).

2.7 Soil Organic Matter and Soil Total Nitrogen

First, TC and TN were measured by rolling 0.2 grams of air-dried and pulverized soil, <0.1 mm, into foil wells, and analyzed on a LECO combustion analyzer (LECO Corporation, St. Joseph, MI). Next, inorganic carbon (IC) was measured with the modified pressure-calcimeter method (Sherrod et al., 2002). We assessed IC in all soil samples with a pH > 7.5 or a C:N ratio of 13 or greater, given those soils likely had substantial IC, which accounted for 79% of all soil

samples. Soils, air-dried and pulverized to a <0.1-mm fine consistency, were weighed into 1-g, 0.5-g, or 0.25-g samples depending on known C:N, >13, >16, >20, respectively. Samples were acidified with 2 mL of 6M HCl, sealed, and allowed to rest for 2 h. The voltage output of the reaction was measured on a pressure transducer and voltmeter at the peak of pressure. A calibration curve, developed from the reaction of $CaCO_3$ and sand, was used to determine IC concentrations which were then subtracted from TC to obtain SOC. This assessment allowed for inference of SOM by multiplying SOC by 1.72, as roughly 58% of the mass of SOM is C (Khatoon et al., 2017).

2.8 Statistical Analysis

The R statistical package (v 4.1.2) was used for all statistical tests in this study (R Core Team 2021). Analysis of variance (ANOVA) was used to test the first hypothesis that crop type will affect PMN and soil enzyme activity (R Core Team 2021). ANOVA was also used to test the second hypothesis, that overwintered crop residue may be associated with increases in POxC. Fisher's LSD, with an alpha level of 0.05 and 95% confidence intervals, was used to compare differences in the response variables of POxC, soil enzyme activity, and PMN, TC, TN, SOC, and residue between fields (de Mendiburu, 2023). Cook's distance with a limit four times the mean was utilized for assessing outliers. Statistical assumptions, such as normality and equal variance, were verified with Residual vs Fitted and Q-Q plots for all models to determine whether any transformations were required to normalize the data (R Core Team 2021). Further, a correlation matrix was created using Pearson's coefficient (r) to test for associations between crop residue and all soil health indicators, including individual soil enzymes, PMN, POxC, SOM, TC, and TN, with an alpha level of 0.10 (Harrell, 2022).

3. Results

3.1 Potentially Mineralizable Nitrogen

PMN ranged from less than 5 mg N·kg soil^{-1} in soils following oilseed to over 20 mg N·kg soil^{-1} following root crops across both study years. PMN was higher in root crop soils than oilseed soils and cereals alone in Year 1 (Figure 2.1; $F_{3,20} = 5.5$, $p < 0.01$) and higher than cereals and dryland legume (yellow pea) in Year 2 (Figure 2.1 $F_{3,20} = 3.26$, $p < 0.05$), but similar to PMN following an intercropped cereal/legume in Year 1 and similar to irrigated legume (soybean) in Year 2.

3.2 SOM Impacts to Soil Enzyme Activity and POxC

SOM was analyzed as a soil characteristic in Year 2 of the study. SOM was higher in Field 2 R-L (i) than all other fields, with 2.8% SOM (Table 2.1; $F_{3,20} = 24.12$, $p < 0.001$). The SOM ranged from 1.2 – 1.9% in dryland soils, and the other irrigated field had 2.1% SOM.

The geometric mean of enzyme activity ranged from a low of 20 mg PNP·kg soil^{-1}·h^{-1} to a high near 50 mg PNP·kg soil^{-1}·h^{-1} (Figure 2.2) Root crop soils were associated with higher soil enzyme activity than all other crop types in Year 1 ($F_{3,20} = 12.05$, $p < 0.001$). However, in Year 2, the pattern of enzyme activity did not persist based on crop type, but instead was linked with the field. Field 2 R-L (i), which had the highest activity rate following root crops in Year 1, was cropped with a legume (soybean) in Year 2, and it retained higher enzyme activity than soils cropped in cereals and other legumes (yellow pea), but the activity was similar to the activity of root crop soils in Field 1 C/L-R (i) ($F_{3,20} = 4.96$, $p < 0.01$). Further, SOM had a positive correlation with enzyme activity, across both study years (Figure 2.3; $r = 0.67$, $p < 0.001$).

Individual enzyme activity also varied across fields (Table 2.3). Arylsulfatase activity was higher in root crop soils than soils following any other crop type in Year 1 ($F_{3,20} = 37.81$, $p < 0.001$), and in Year 2, arylsulfatase activity was higher in soils following root crops than cereals or dryland legumes (yellow pea), but similar to irrigated legumes (soybean) ($F_{3,20} = 15.3$, $p < 0.001$). Total phosphatase activity, the summation of acid and alkaline phosphatases, was similar in all soils, regardless of crop type, across both years (Year 1, $F_{3,20} = 2.82$, $p > 0.05$; Year 2, $F_{3,20} = 2.26$, $p > 0.1$). β-glucosidase activity was lower following cereals alone than all other crop types in Year 1 ($F_{3,20} = 6.11$ $p < 0.01$). However, in Year 2, B-glucosidase activity was higher following irrigated legume soils (soybean) compared to all other crop types ($F_{3,20} = 12.97$, $p < 0.001$). In Year 1, β-glucosaminidase activity did not differ between fields ($F_{3,20} = 2.32$, $p = 0.1$). By Year 2, β-glucosaminidase activity was higher following irrigated legumes (soybean) and cereals than other crop types ($F_{3,20} = 8.42$, $p < 0.001$).

The POxC concentration ranged from 400 mg C·kg soil^{-1} to nearly double at 800 mg C·kg soil^{-1} across all fields in both study years. Soil POxC concentrations were higher in soils following oilseeds than cereals or cereal-legume intercrop in Year 1, but similar to the POxC in root crop soils (Figure 2.4; $F_{3,20} = 4.1$, $p < 0.05$). However, in Year 2 of the study, POxC was higher following irrigated legume (soybean) and cereals than in soils following dryland legumes (yellow pea) and root crops (Figure 2.4; $F_{3,20} = 3.86$, $p < 0.05$). Soil POxC was not strongly correlated with SOM ($r = 0.24$, $p = 0.1$).

3.3 Soil C and N

TC in the upper 15 cm ranged from 1.99 – 2.15% in irrigated soils and from 0.97 – 1.80% in dryland soils (Table 2.4). Irrigated soils had higher TC in both study years than dryland soils

(Year 1, $F_{3,20} = 179.6$, $p < 0.001$; Year 2, $F_{3,20} = 74.06$, $p < 0.001$). TN in the upper 15 cm ranged from 0.05% to nearly 0.15% across the fields (Table 2.6). TN was higher in root crop and oilseed soils than cereals and cereal-legume soils in Year 1 ($F_{3,20} = 8.01$, $p < 0.001$). TN was higher following irrigated legumes (soybean) than dryland legumes (yellow pea) or cereals, but similar to root crop soils in Year 2 ($F_{3,20} = 10.27$, $p < 0.001$).

3.4 Overwintered Crop Residue

The quantity of crop shoot residue that overwintered to the beginning of the next growing season varied across fields and by crop type, ranging from roughly 1.1 Mg ha^{-1} after sugar beets to nearly 12 Mg ha^{-1} after winter wheat (Table 2.5). At the beginning of the 2022 growing season, Year 1 of the study, the field that was growing only cereals retained a higher amount of surface crop residue over winter than root crops and oilseeds but was similar to the cereal-legume intercrop ($F_{3,20} = 7.43$, $p < 0.001$). By the beginning of the 2023 growing season and Year 2 of the study, the field growing dryland legumes (yellow pea) retained more crop residue than fields following root crops and irrigated legumes (soybean) but was similar to the field growing cereals ($F_{3,20} = 8.43$, $p < 0.001$).

Root residues in the upper 20 cm of the soil profile were analyzed in the beginning of Year 1 and ranged from 0 – 0.36 Mg ha^{-1}. The remaining root residue mass in soil was greater following an irrigated cereal-legume intercrop than dryland cereals alone, oilseeds, and root crops (Table 2.6; $F_{3,20} = 5.54$, $p < 0.01$). Because root residue biomass was minimal from all fields, it was only assessed in the first year.

3.3 Surface Crop Residues and Soil Biological Response

Overwintered crop residue correlations with soil biological response functioned differently between the two years of this study (Table 2.7). In Year 1, seven biological parameters were significantly correlated with surface residue that remained on the soil surface by the beginning of the next growing season. The quantity of residue was moderately correlated with three of five soil enzymes. Arylsulfatase was negatively correlated with crop residue quantity ($r = -0.50$, $p < 0.01$). Phosphatase enzyme activity responded differently to crop residues, where acid phosphatase was positively correlated ($r = 0.53$, $p < 0.01$) and alkaline phosphatase was negatively correlated ($r = -0.58$, $p < 0.01$). Further, crop residues had weak correlations with aspects of soil nutrient pools in Year 1. Residue was negatively correlated with TC ($r = -0.50$, $p < 0.01$), TN ($r = -0.37$, $p < 0.1$), POxC ($r = -0.35$, $p < 0.1$), and SOM ($r = -0.48$, $p < 0.05$). However, in Year 2, PMN was the only soil biological response correlated to crop residues ($r = -0.37$, $p < 0.1$), likely driven by low residue associated with root crops.

4. Discussion

Throughout the growing season, plants contribute to soil biological activity in many ways, and surface crop residue only accounts for one element of a much more complicated system of plant-soil feedbacks (PSFs). PSFs are defined as an ecological process where plants leave biotic and abiotic legacies in soil, which can ultimately affect subsequent plant growth (Koyama et al., 2022). This has been demonstrated in agricultural settings with variation in crop yields depending on crop sequence (Benitez et al., 2017). Crops differ in their root architecture and rhizodeposition, which can have major impacts on biogeochemical cycling (Benitez et al., 2017). Root crops like sugar beet, where the substantial belowground biomass is harvested, leave

little root residue in the soil; however, the large root bulb likely contributes many resources to soil through its root exudates during the growing season. On the other hand, cereals have less root biomass during the growing season, but that biomass is relatively undisturbed during harvest.

4.1 Sugar Beet May Drive Mineralizable N

PMN was the only soil health indicator that had a notable link with specific crop type across both study years, namely sugar beet. One possible reason for higher PMN in soils following sugar beet is the high N content of sugar beet leaves, which are severed from the root redistributed on the soil surface, then during root harvest, buried under a layer of soil. Sugar beet residues can contribute between 100-150 kg $N \cdot ha^{-1}$ to the soil (Whitmore & Groot, 1997). The sugar beet leaves, which are composed of 4-6% N with a C:N around 11 during the growing season (Varga et al., 2022; Whitmore & Groot, 1997), but falling to around 1.5% N with a C:N around 30 at senescence (Eslami et al., 1988), can rapidly decompose, ultimately increasing mineralization rates and mineral N (Ranaivoson et al., 2017). This was evident by increased PMN following sugar beet in two separate fields over the two-year study period.

Sugar beets also have a higher N requirement than other common crops grown in Montana, except for high protein yielding wheat varieties (Table 2.8). To achieve desired belowground root biomass yields and sucrose content, sugar beets are fertilized at an N rate much higher than legume and oilseed crops, but only slightly higher than cereal crops for average yield expectations in Montana (Jacobsen et al., 2003). In this study, sugar beets were fertilized at an N rate at least six-fold higher than legumes, 4x than oilseeds, and at least 4x than cereals (Table 2.9). The low fertilizer rates of legumes, oilseeds, and cereals on this farm are due

to N credits from prior legumes in these crop rotations. Increased N fertilizer rates have been associated with lower PMN in other studies on cereal and legume crop rotations in Montana (O'Dea et al., 2015). Therefore, it is likely that the increased fertilizer requirement of sugar beets may only affect the PMN pool indirectly by increasing overall biomass and crop residue inputs of the high-quality sugar beet leaves.

4.2 Enzyme Activity: A Function of SOM and Soil Texture

Extracellular enzyme activity in these soils did not follow a clear pattern based on the previous crop, but it did follow a pattern based on field. For both study years, the geometric mean of enzyme activity was highest in Field 2 R-L (i), which was cropped in sugar beet for the first study year and a legume, soybean, in the second year. Two notable observations about the soil characteristics of Field 2 R-L (i) are its higher SOM content and higher clay content than other fields in this study. This field had an average of 2.8% SOM, nearly a full percentage point greater than the other fields. It was also classified as a clay loam, with 27% clay, compared to the sandy loam and loam soils found in the other fields.

Both SOM and clay provide increased surface area to bind extracellular enzymes, stabilizing the enzymes within the soil matrix (Burns et al., 2013; Wallenstein & Weintraub, 2008). These immobilized enzymes often have reduced activity levels when compared to their free-living counterparts, but their stability results in persistent activity for prolonged time periods. Therefore, it is likely that SOM, and potentially soil texture with the increased water holding capacity of clay, are driving the increased geometric mean of enzyme activity observed in Field 2 R-L (i), demonstrated by a strong positive correlation between enzyme activity and SOM (Figure 2.3). And while crop residue is a known driver of building SOM, soils may need

more than five years to show accumulation of SOM and nutrient pools from residues in diversified crop rotations and to increase biological activity (Blanco & Lal, 2023). Further, to detect effects on enzyme activity linked to crop type, it may be imperative to conduct crop sequence research on soils with comparable SOM. This would eliminate the confounding variable driving enzyme activity responses observed in this study.

4.3 Overwintered Residue Not a Predictor of Soil Response

Ultimately, the quantity of crop residue that remained on the soil surface until the beginning of the next growing season was not a robust predictor for soil biological indicators, unlike our initial hypothesis. We found no clear relationship between residue quantity and any of the biological soil health indicators assessed that persisted across both study years. Some soil enzymes, POxC, TC, TN, and SOM were negatively correlated with crop residue in the first year, suggesting that decomposed residue, resulting in lower quantities of residue that remained on the surface by spring of the next year, had contributed to increased biological activity. However, none of these biological responses maintained a consistent relationship with overwintered crop residue across study years. This could be due to differences in abiotic conditions between years, like temperature and moisture.

During the first growing season of this study, this region of Montana experienced extreme drought conditions which greatly impacted crop yields, and inherently the crop residue that remained after the growing season. The nearest weather station in Hysham, MT, 20 miles west of the site, reported 81 mm of precipitation from May through August of 2021, and only 37 mm of precipitation excluding the last half of August, after most crops were harvested. In the second study year of 2022, the Hysham weather station reported 135 mm of precipitation for the same 4

month period (Southern Agricultural Research Center 2022). While irrigated crops are not impacted to the same degree by natural precipitation amounts and patterns as dryland crops, the 2021 drought did contribute to lower water availability from the lack of precipitation. The wheat grain yield was only 1.0 Mg ha^{-1} in 2021, which would have produced 1.7 Mg ha^{-1} of residue using typical Harvest Index, and perhaps twice that knowing it was a drought year. Therefore, it was surprising and noteworthy that we measured 12 Mg ha^{-1} of overwintered residue, suggesting that residue from previous crops had not decomposed.

Residue decomposition slowly releases nutrients to soil, meaning that a decrease in overwintered crop residues should be associated with increases in soil nutrient content, if most of the residues have decomposed. However, depending on the chemical composition of the residues, this may only occur over longer timescales (Ranaivoson et al., 2017). In the short term, such as within the two-year timeframe of this study, nutrients can become immobilized as microbes decompose the crop residues, meaning high quantities of overwintered residue inputs could inhibit soil nutrients the following year (Ranaivoson et al., 2017). Some high-residue crops with high C:N, like cereal crops, do not readily decompose. N can become immobilized in the process of further decomposition, resulting in residues remaining on the soil surface for years, and a delayed response to residue additions. This could explain the relationships observed in this study, where quantity of overwintered crop residue did not correlate with soil biological indicators of nutrient availability, PMN and enzyme activity, over the two-year study period.

Root crops like sugar beet contribute high quality residues in a unique way to the soil compared to other crop types. Before sugar beet harvest, the leaves and upper portion of the root bulb of the beet are clipped and left in the field. During harvest, the root bulbs are extracted from

the ground, which leaves the high quality, low C:N residues covered in a thin layer of soil at the surface. This provides a protected and wetter environment for rapid decomposition of the beet residues. Because of this, we calculated correlations between overwintered crop residue and soil biological parameters both with and without root crops. Ultimately, this did not change the relationships observed between residue quantity and soil biological parameters in either study year, with the same weak negative relationships persisting with or without root crops.

Crop residue quantity is critical for maintaining soil ecosystem services. In the more humid corn belt region of the Great Plains, a minimum of 3.9 ± 2.18 Mg ha^{-1} of crop residues are required to maintain SOC (Johnson et al., 2014). More conservative estimates indicate that at least 5-12 Mg ha^{-1} of residues are needed each year to sustain SOC (Blanco & Lal, 2023; Wilhelm et al., 2007), though these studies were focused in the central Great Plains. Studies in the semi-arid Canadian NGP have reported 2.4 Mg C ha^{-1} y^{-1} are needed to maintain SOC pools (Shrestha et al., 2013), or roughly 6.0 Mg ha^{-1} of residue returned to the soil, assuming 40% C in crop residue (Stewart, 1993). Montana based studies have found that 2.6 Mg C ha^{-1} in aboveground residues, root biomass, and rhizodeposits are required to maintain SOC (Engel et al., 2017), or roughly 6.5 Mg ha^{-1} of crop residues each year. Below this threshold, soil C diminishes, depending on abiotic factors.

4.4 Implications & Future Research Directions

Overwintered crop residue did not stand alone as a predictor of the soil biological response associated with PMN, soil enzyme activities, and POxC at the beginning of the next growing season. However, the relationship between crop residues and soil biological activity may be better explained by measuring crop residues at harvest and those remaining the following

spring, to get an understanding of the potential quantity of residue that may have decomposed, contributing to nutrient release and SOM building. Early in the decomposition process, some C is lost to the atmosphere, with legumes exhibiting the highest CO_2 emissions per gram of C added, followed by oilseeds then cereal crops (Ntonta et al., 2022). The quality, or C:N, of residues plus their biochemical compositions controls decomposition rates (Cookson et al., 1998), meaning that different crop types will have varying rates of decomposition, impacting the timeframe where crop residues impact soil microbial activity. Understanding the proportion of residues that decompose from harvest to the following spring may help to predict biological responses to crop residue, and the timeframe when those responses can be expected, which may differ based on residue quality and composition.

This research was a two-year observational study that took place on a farm in eastern Montana with the aim of identifying how crop sequences with various crop types and their residues impact biological soil health indicators. Future research that aims to understand the dynamic relationship between crop types, residue, and soil health will need to incorporate more residue sampling at various times throughout the year, increase the number of research sites across the region, and include full rotations to understand the longer lasting impacts of crop sequence. Further, it may be beneficial to include more comparisons of the impacts of various crop types in both dryland and irrigated systems to understand how water regimes affect both crop residue biomass and subsequent decomposition rates.

Table 2.1 Soil characteristics for a 0 – 15 cm depth, and crops grown in the four fields sampled in Forsyth, MT, on May 16, 2022, and May 1, 2023, averaged across both sampling years.

Field	1 C/L-R (i)	2 R-L (i)	3 O-L (d)	4 C-C (d)
Texture	Loam	Clay loam	Loam	Sandy loam
Clay (%)	17.6	27.0	16.5	14.5
pH	7.9	8.0	8.1	6.2
SOM (%)	2.1	2.8	1.9	1.5
Irrigation status	Irrigated	Irrigated	Dryland	Dryland
Year 1 Crop	Millet/Faba bean	Sugar beet	Sunflower	W. wheat
Year 2 Crop	Sugar beet	Soybean	Yellow pea	S. wheat

Table 2.2 Yield, in Mg ha^{-1}, by field and crop for both study years.

	----------Year 1----------		----------Year 2----------	
Field	Crop	Crop yield	Crop	Crop yield
1 C/L-R (i)	Millet/Faba bean*	4.48	Sugar beet	70.12
2 R-L (i)	Sugar beet	68.26	Soybean	3.05
3 O-L (d)	Sunflower	0.54	Yellow pea	2.13
4 C-C (d)	W. wheat	1.03	S. wheat	2.73

*Faba bean crop failed, only millet crop yield reported.

Table 2.3 Enzymatic activity (mg PNP g soil^{-1} hr^{-1}) of five soil enzymes (ß-glucosidase, ß-glucosaminidase, acid and alkaline phosphatases, and arylsulfatase) and the geometric mean of all enzyme activities for four fields following different crops for two years.

	Acid Phosphatase		Alkaline Phosphatase		Total Phosphatase		Arylsulfatase		ß-glucosidase		ß-glucosaminidase		Geometric mean	
Field	Yr 1	Yr 2	Yr 1	Yr 2	Yr 1	Yr 2	Yr 1	Yr 2	Yr 1	Yr 2	Yr 1	Yr 2	Yr 1	Yr 2
1 C/L-R (i)	12.7^{c}	32.4^{b}	115.5^{a}	135.6^{a}	128.2	167.9	31.2^{b}	29.9^{ab}	44.4^{a}	37.3^{b}	12.3	2.7^{b}	29.5^{b}	25.7^{ab}
2 R-L (i)	39.2^{b}	26.0^{b}	144.7^{a}	135.8^{a}	183.9	161.9	47.8^{a}	36.4^{a}	53.1^{a}	51.4^{a}	13.2	8.1^{a}	44.4^{a}	34.8^{a}
3 O-L (d)	11.6^{c}	26.8^{b}	125.8^{a}	136.7^{a}	137.4	163.6	32.7^{b}	28.8^{b}	46.7^{a}	33.5^{b}	6.8	2.2^{b}	27.7^{bc}	21.1^{b}
4 C-C (d)	116.0^{a}	100.2^{a}	14.2^{b}	30.7^{b}	130.2	128.1	9.6^{c}	13.5^{c}	26.4^{b}	18.5^{c}	10.5	6.3^{a}	20.4^{c}	19.2^{b}
p-value	<0.01	<0.01	<0.01	<0.01	0.1	0.1	<0.01	<0.01	<0.01	<0.01	0.1	<0.01	<0.01	<0.01
F-stat $_{3, 20}$	59.84	29.18	19.75	20.42	2.82	2.26	37.81	15.3	6.11	12.97	2.32	8.42	12.05	4.96

Different letters indicate significant differences between fields within that year ($\alpha = 0.05$).

Table 2.4 Total C (%), total N (%), C:N, and SOC (%) of soils, averaged across four fields, following different crop types.

	Total C		Total N		SOC
Field	Yr 1	Yr 2	Yr 1	Yr 2	Yr2
1 C/L-R (i)	2.12[a]	2.12[a]	0.09[b]	0.09[ab]	1.21[b]
2 R-L (i)	1.99[b]	2.15[a]	0.14[a]	0.10[a]	1.61[a]
3 O-L (d)	1.80[c]	1.67[b]	0.12[a]	0.08[b]	1.12[b]
4 C-C (d)	0.97[d]	0.97[c]	0.10[b]	0.06[c]	0.90[c]
p-value	<0.01	<0.01	<0.01	<0.01	<0.01
F-stat $_{3, 20}$	179.6	74.06	8.01	10.27	24.12

Different letters indicate significant differences between fields within that year ($\alpha = 0.05$).

Table 2.5 Dry weight of overwintered surface crop residues, by field and crop, estimated in Mg ha^{-1} for both study years.

	----------Year 1----------		----------Year 2----------	
Field	Crop	Crop residue	Crop	Crop residue
1 C/L-R (i)	Millet/Faba bean	7.13[ab]	Sugar beet	1.14[c]
2 R-L (i)	Sugar beet	3.56[bc]	Soybean	3.32[bc]
3 O-L (d)	Sunflower	1.52[c]	Yellow pea	7.38[a]
4 C-C (d)	W. wheat	11.97[a]	S. wheat	5.09[ab]
p-value		<0.01		<0.01
F-stat $_{3,20}$		7.43		8.43

Different letters indicate significant differences between fields within that year ($\alpha = 0.05$).

Table 2.6 Dry weight of root residues for a 0 – 20.5 cm depth, by field and crop, estimated in Mg ha^{-1} for Year 1 of study.

Field	Crop	Root residue
1 C/L-R (i)	Millet/Faba bean	0.36[a]
2 R-L (i)	Sugar beet	0.00[b]
3 O-L (d)	Sunflower	0.04[b]
4 C-C (d)	W. wheat	0.04[b]
p-value		<0.01
F-stat $_{3,20}$		5.54

Different letters indicate significant differences between fields within that year (α= 0.05).

Table 2.7 Pearson correlation coefficients (r) of soil biological responses with aboveground crop residues that overwintered from the previous year for both study years across four fields.

Soil Biological Response	Year 1	Year 2
Acid Phosphatase	-0.53***	-0.03
Alkaline Phosphatase	0.58***	0.03
Arylsulfatase	-0.50**	-0.10
ß-glucosidase	-0.31	-0.25
ß-glucosaminidase	0.32	-0.17
Geometric Mean	-0.25	-0.20
PMN	-0.05	-0.37*
POxC	-0.35*	0.24
TC	-0.50**	-0.32
TN	-0.37*	-0.21
SOM	-0.48**	-0.24

p-value *** <0.01; **<0.05; *<0.1

Table 2.8 Montana crop N requirements, range is determined by yield potential.

Crop	N requirement (lbs ac^{-1})
Pea/lentil	15-40*
Sugar beet	144-288
Sunflower	50-125
Wheat	99-300**
Millet	52-107

Indicator that N range includes both *irrigated and dryland crop requirements or **spring and winter high protein varieties. (Jacobsen et al., 2003).

Table 2.9 N additions through urea, in kg ha^{-1}, by field and crop for both study years.

	----------Year 1----------		----------Year 2----------	
Field	Crop	N applied	Crop	N applied
1 C/L-R (i)	Millet/Faba bean	34	Sugar beet	202
2 R-L (i)	Sugar beet	209	Soybean	0
3 O-L (d)	Sunflower	49	Yellow pea	0
4 C-C (d)	W. wheat	0	S. wheat	45

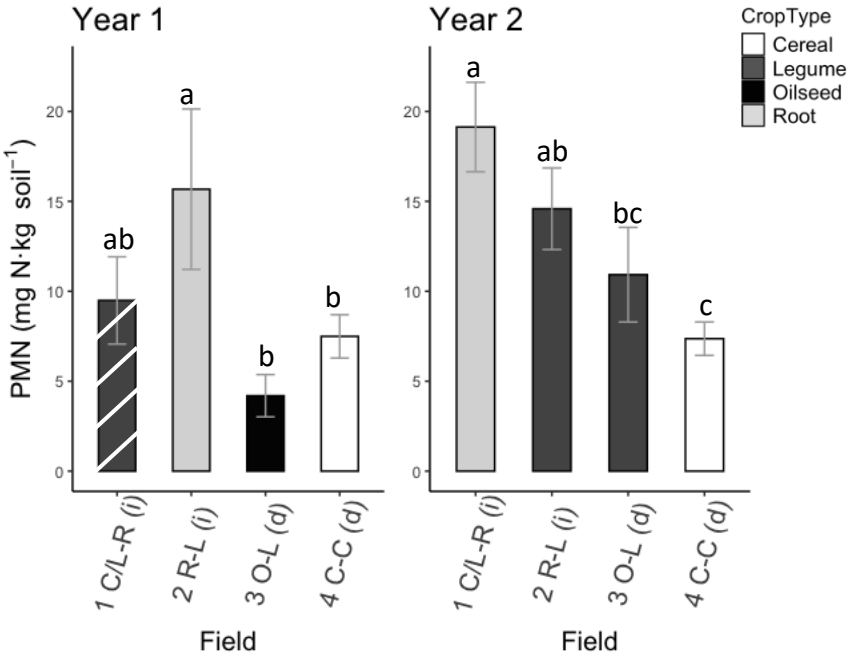

Figure 2.1 Potentially mineralizable nitrogen (mg N·kg soil^{-1}) by field and crop type for both study years. Stripes indicate intercropped crop types of legumes and cereals. Different letters indicate significant differences between fields within that year ($\alpha = 0.05$).

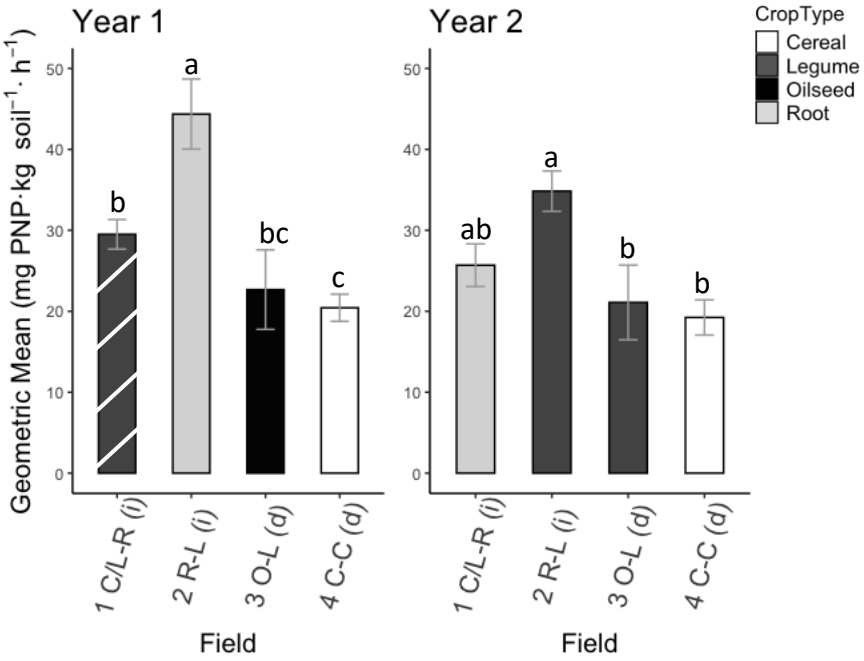

Figure 2.2 Geometric mean of enzyme activity (mg PNP·kg soil^{-1}·h^{-1}) by field and crop type for both study years. Stripes indicate intercropped crop types of legumes and cereal. Different letters indicate significant differences among fields within that year ($\alpha = 0.05$).

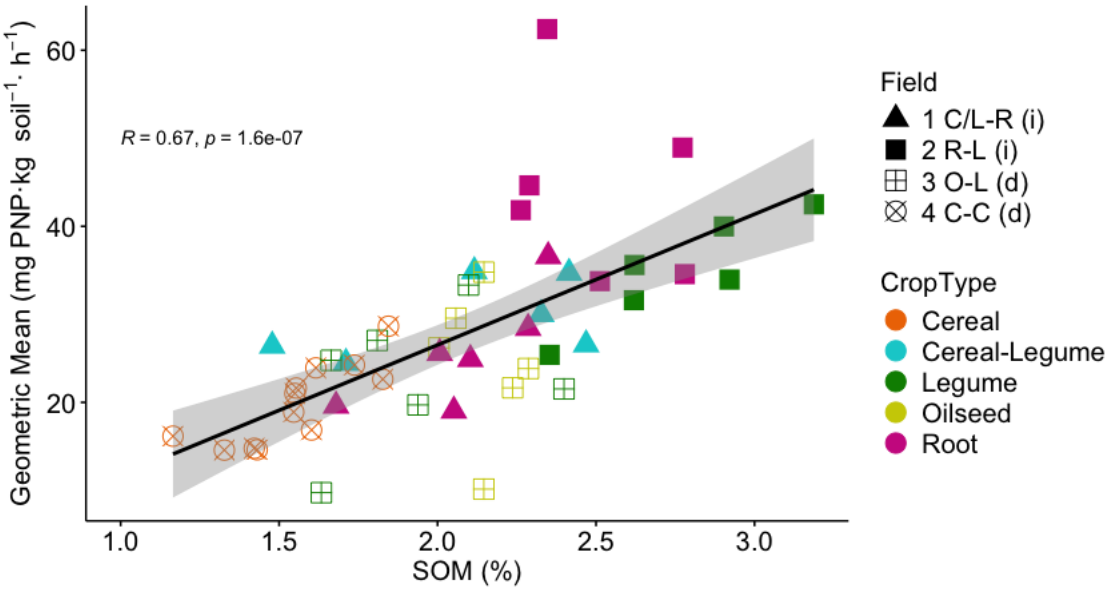

Figure 2.3 Correlation of soil organic matter vs. geometric mean of enzyme activity across both study years, represented by both crop type and field.

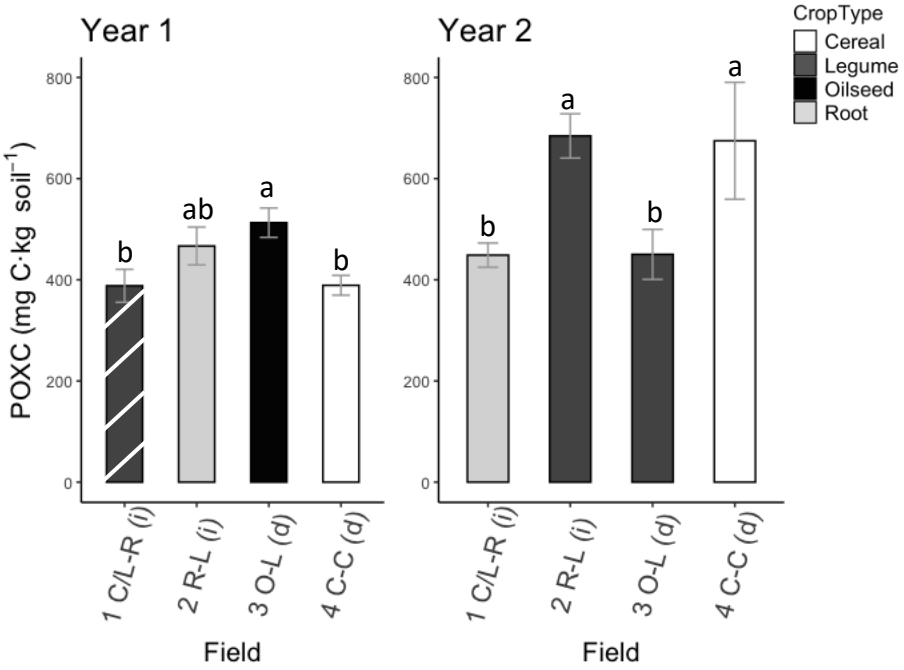

Figure 2.4 Permanganate oxidizable carbon (mg C·kg soil^{-1}) by field and crop type for both study years. Stripes indicate intercropped crop types of legumes and cereal. Different letters indicate significant differences among fields within that year ($\alpha = 0.05$).

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CHAPTER THREE

MEASURING THE SOIL HEALTH GAP BETWEEN CROPLAND AND UNCULTIVATED SOILS OF SEMI-ARID AGROECOSYSTEMS IN MONTANA

Abstract

The Northern Great Plains is a mosaic of productive croplands, grasslands, forest, pasture, and shrublands. However, the conversion of grassland ecosystems to crop production has had major impacts to ecosystem function, nutrient cycling capacity, and soil health. We conducted research on two farms in Montana to compare soil health metrics between cropped and adjacent grassland soils. The primary goal of this research was to quantify the soil health gap and identify potential soil health benchmarks based on uncultivated lands to understand the potential capacity of soil biological function in a specific agroecosystem. Further, we set out to understand the greater implications of acidification on soil biological activity by assessing paired acid and neutral pH soils in a single field. We assessed soil biological indicators of extracellular enzyme activity (five total) and potentially mineralizable nitrogen (PMN), as well as permanganate oxidizable carbon (POxC) and soil organic matter (SOM) in ten paired cropped and uncultivated soils and six paired acid and neutral pH soils. The geometric mean of five enzyme activities was 59% lower on average in cultivated soils when compared to their uncultivated counterparts (average difference of 43.4 mg PNP·kg soil^{-1} h^{-1}; $t_9 = -5.49$, $p < 0.001$). Cultivated soil PMN was 51% lower on average than the uncultivated soil (15.3 mg N·kg soil^{-1}; $t_9 = -3.46$, $p < 0.01$). Soil POXC was 39% lower from uncultivated to cropped soils (338.2 mg C·kg soil^{-1}; $t_9 = -2.93$, $p <$

0.05). SOM was also 39% lower in cultivated soils but was more stable year-to-year than biological indicators (1.3%; $t_9 = 4.22$, $p < 0.01$). Acidified soils (pH < 5) had 42% lower geometric mean of enzyme activity of four enzymes, not including arylsulfatase, than areas within the same field with near neutral pH that were growing alfalfa (17.9 mg PNP·kg soil^{-1} h^{-1}; $t_5 = 2.52$, $p < 0.05$), but with higher SOM by 12%, on average, in acidified soils (0.27%; $t_5 = 3.43$, $p < 0.05$). There were no differences in PMN or soil POxC between different pH levels. Overall, we found that cultivation resulted in an average of 45% lower soil health across all parameters tested when compared to uncultivated sites, but soil acidification was not consistently lower across all soil health parameters tested compared to neutral pH soils, except for three enzyme activities.

1. Introduction

The Northern Great Plains (NGP) is a mosaic of productive croplands, grasslands, forest, pasture, and shrublands. Most of the remaining intact grasslands exist in the western, semi-arid region of the Great Plains, and some are unsuitable for crop production due to abiotic constraints such as topography and rockiness (Smart et al., 2021). However, the rest of these persisting grasslands are at risk of conversion to croplands for a variety of reasons, including biofuel production, meeting the global food demand, and greater economic opportunities. In association with high crop prices in the beginning of the 21st century, various land cover spatial analyses have estimated that up to two million ha of grasslands, including some Conservation Reserve Program (CRP) grasslands, were transitioned to croplands between 2006 and 2015 across the U.S. (Johnston, 2014; Lark et al., 2015; Lark et al., 2020; Reitsma et al., 2015; Wright et al., 2017; Wright & Wimberly, 2013) with the majority of this land use change occurring in areas of

the NGP (Lark et al., 2020; Reitsma et al., 2015). Thus, the NGP is considered a highly endangered ecoregion due to increasing shifts from grassland to agricultural landscapes (Fred et al., 2004). In Montana, there are just under 19.3 million ha of grasslands (range and pasture) and 6.7 million ha of croplands, approximately 51.1% and 17.8% of the state's land area, respectively (Bigelow & Borchers, 2017). Ultimately this means that a sizeable portion of Montana's land area, depending on abiotic constraints, may be subject to conversion to crop production in the future as global food demand increases. This land use change to annual cultivation could have major implications for ecosystem function, nutrient cycling potential, and soil health.

Native grasslands provide ample ecosystem services, including forage for livestock, habitat for wildlife, increased carbon (C) storage and sequestration, and even cultural values (Bengtsson et al., 2019; Smart et al., 2021). These landscapes provision ecosystem services that are unique to the native prairie that often cannot be recreated in cultivated lands. Land use changes not only alter the plant diversity and composition aboveground, but also the associated soil properties and microbial communities of that soil (Jangid et al., 2010). While grassland soils are resilient to external disturbances such as major climate events, cultivation results in changes to a soil's physical, chemical, and biological properties, impacting soil function (Gregory et al., 2009). There are well documented losses of soil organic carbon (SOC) and soil structure with land conversion to crop production (DeLuca & Zabinski, 2011; Haas & Evans, 1957; Liang et al., 2023; Malo et al., 2005; Mann, 1986). Specifically, NGP soils have experienced significant decreases in SOC, with losses around 42 ± 11% in studies throughout the region (Haas & Evans, 1957; Liebig et al., 2009). When lands with native perennial grassland communities are converted to annually cultivated croplands, the density and depth of the rooting system are

greatly reduced, impacting the physical structure of soils (Reitsma et al., 2015). To understand the impacts of land use change and crop production on soil function, it's imperative to quantify the decline in soil health associated with cultivation.

The US Environmental Protection Agency has set benchmarks and quality standards to protect both water and air resources (EPA, 2024). While the Natural Resource Conservation Service (NRCS) has been tasked with ensuring soil conservation, there are few agreed upon metrics, no quantifiable targets, and minimal regulations to promote the protection of soil quality and health (Amsili et al., 2023). Identifying and defining quantifiable soil health targets could support climate mitigation efforts and broader conservation goals, while aiding producers with tangible soil health goals to work toward. Soils are very heterogenous across the landscape, shaped by both biotic and abiotic conditions. Therefore, it is critical to identify a relativized soil health benchmark, one that links the potential capacity and function of a soil within a specific agroecosystem and climate regime.

The Soil Health Gap, introduced by Maharjan et al. (2020), is a concept that explores the difference in soil health indicators between undisturbed native soils with cropped soils that share climate characteristics and soil properties. Determination of a soil health benchmark, localized to a specific agroecosystem, describes both the gap in soil health parameters between managed croplands and uncultivated soils, while also delineating the degree of soil degradation (Maharjan et al., 2020). This information can then be utilized by farmers and producers to set potential and realistic soil health goals relative to their specific soil. Much of the research on the gap in soil health between native grassland ecosystems and annually cropped soils has focused solely on SOC and carbon sequestration potential (Amsili et al., 2023; Beniston et al., 2014; DeGryze et

al., 2004; Houghton, 1995; Kaye et al., 2005). However, the C cycle only represents one fraction of dynamic soil systems.

Research on soil health often focuses primarily on the chemical and physical properties of a soil, which fails to provide a complete picture of the status of soil function, and the entire soil-plant-ecosystem relationship (Raiesi & Salek-Gilani, 2020). Biological soil health indicators help to comprehensively determine soil microbial community characteristics and their influence on biogeochemical cycles (Bhaduri et al., 2022). Further, they are particularly sensitive to changes in land use and land management (Steinberger et al., 2022). We selected four soil health indicators to explore biological responses to cultivation compared to uncultivated lands: soil extracellular enzyme activity, potentially mineralizable nitrogen (PMN), permanganate oxidizable carbon (POxC), and soil organic matter (SOM). These four indicators allow for assessment of nutrient cycling capacity through soil enzyme activity, plant available N via PMN, labile C dynamical interaction with the soil matrix from soil POxC, and the physical, chemical, and biological function through SOM. The oxidation of POxC mimics the microbial decomposition of SOM and is the C indicator most representative of the labile C pool (Bongiorno et al., 2019), though recent research suggests that it is also reflects the soil mineral fraction of the C cycle (Woodings & Margenot, 2023).

The primary objective of this study is to quantify the soil health gap on two farms in Montana, representing typical agroecosystems of the region, by comparing biological soil function in cropped soils to nearby uncultivated soils that have some influence from livestock grazing and wildlife browsing to varying degrees. Because agricultural production impacts soil physical, chemical, and biological traits, we hypothesized that cultivation would decrease soil

health parameters, evident by decreases in indicators such as soil extracellular enzyme activity, PMN, POxC, and SOM. Through a paired study design, we can directly compare soil health metrics in cropland soils to adjacent uncultivated soils. Ultimately, this research aims to inform producers about the maximum attainable soil health values for their specific lands and provide a realistic soil health goal to work toward through conservation-focused land management.

In addition to impaired biological activity, changes in soil chemistry can impact soil health. In Montana and across the NGP, acidification is a growing issue of serious concern for producers (Reeves & Liebig, 2016). Cropping systems require the use of N fertilizer, often composed of ammoniacal N, which includes urea. Though N fertilizers themselves are not acidic, their inputs to soil can be acid forming. Specifically, through the nitrification of ammonium, NH_4^+, to the plant available N form of nitrate, NO_3^-, H^+ ions are generated, resulting in decreased pH (Schroder et al., 2011). This is especially prevalent in the upper depths of the soil profile in no-till systems because of a lack of soil mixing (Wicks et al., 1988). While no-till systems may be better for retaining soil moisture and SOM, they concentrate low pH in the upper portion of the plant rooting zone, resulting in prevalent pH issues across the region (Tarkalson et al., 2006).

To build on the understanding of the soil health gap when transitioning grassland soils to cultivation, we assessed the implications of N fertilization, to the point of acidification, by quantifying the additional decline in soil health under acid conditions. Soil biological activity is optimized at a near-neutral pH, depending on the organism; however, it is greatly reduced below pH 5 (Rousk et al., 2009). Therefore, our second objective in this study was to assess how biological indicators of soil health in acidified soils ($pH < 5$) compared to neutral pH soils within the same field. We hypothesized that all biological soil health indicators, including enzyme

activity, PMN, POxC, and SOM, would be lower in acidified soils when compared to adjacent areas of near neutral pH with perennial plant cover.

2. Methods

2.1 Site Description

This study was conducted on two farms, the first outside of Forsyth, Montana (46°15’54.33” N, 106°52’09), along the banks of the Yellowstone River. This region of southeastern Montana receives 374 mm of precipitation annually, on average, with an average air temperature of 8.2 °C (Western Regional Climate Center, 2010a), typical of a cold semi-arid climate type. The farm is no-till, with both dryland and irrigated crop production and a diverse rotation of cereals for grain and seed production, legumes, oilseeds, and root crops across its 2,307 hectares. Specifically, the crops cultivated in the fields assessed during this study were winter and spring wheat (*Triticum aestivum L.*), foxtail millet (*Panicum italicum L.*), lentil (*Lens culinaris Medic.)*, yellow pea (*Pisum sativum L.*), faba bean (*Vicia faba L.*), sunflower (*Helianthus annuus L.*), and sugar beets (*Beta vulgaris L.*). The soil at the Forsyth study site is classified as a frigid, Aridic Ustifluvent loam (Soil Survey Staff 2022). Soil characteristics differed among fields (Table 3.1).

The second study site is east of Fort Benton, Montana (47°47'25.07" N, 110°31'23.63" W), near the upper Missouri River. This area of north central Montana receives an average of 335 mm of precipitation annually, with a 7.4 °C average air temperature (Western Regional Climate Center, 2010b), typical of a cold, semi-arid climate type. This farm is managed no-till with dryland crop rotations including legumes, cereals, and perennials across 1,215 hectares. Crops included in this study were yellow pea (*Pisum sativum L.*), spring wheat (*Triticum*

aestivum L.), alfalfa (*Medicago sativa L.*), and intermediate wheatgrass (*Thinopyrum intermedium (Host) Barkworth & Dewey*). The soil at the Fort Benton study site is a frigid, Vertic Argiustoll loam (Soil Survey Staff 2022). Soil characteristics differed among fields (Table 3.1).

2.2 Experimental Design

The primary goal of this study was to assess and compare soil biological traits between agricultural soils and nearby uncultivated soils, where uncultivated lands were often grazed and used as pasture. This study employs a paired design, where for the first objective, soil samples were randomly located and composited from a cropped field to represent cultivated soils, and then compared to randomly located and composited samples from an adjacent uncropped area, referred to as uncultivated soils. The criteria used for selecting uncultivated sites were areas: 1) with a dominantly intact perennial plant community with minimum bare soil; 2) away from fence lines or field margins; and 3) with proximity, within 15-100 m, to agricultural soils of the same soil series. Uncultivated site selection was also informed by collaborating producers to ensure that the soils had not been cultivated in recent history, or at least the past two generations of farming. Many of the uncultivated sites were used for seasonal grazing and not in crop production due to complicated microtopography or odd shaped areas near the edges of cropped fields.

At the study site in Forsyth, four fields and their uncultivated counterparts were sampled over two years (Table 3.1). The irrigated crop sequence in the two years preceding sampling at the Forsyth farm included millet-faba bean (M-FB) in Year 1 and faba bean-sugar beet (FB-SB) in Year 2 on one field. One lowland dryland field located near the irrigated fields was in a sequence of lentil-sunflower (L-S) in Year 1 and sunflower-pea (S-P) in Year 2. The two dryland

upland fields are in a longer rotation of winter wheat-spring wheat-lentil-yellow pea and have been cropped under this rotation for the last 30 years, with the first field in a winter wheat-spring wheat (WW-SW) rotation in Year 1 and spring wheat-winter wheat (SW-WW) in Year 2, and the second field in a pea-winter wheat (P-WW) rotation only sampled in Year 2.

At the Fort Benton study site, one field with an adjacent uncultivated coulee was selected for assessment (Table 3.1). This agricultural field was managed under the NRCS CRP, meaning it was growing perennial grasses for 30 years up until 15 years ago. The field was initially managed organically, but the recent 7 years preceding this study was conventionally managed with no-till. Currently, the field is divided into strips of yellow pea-spring wheat (P-SW) and third-year perennial intermediate wheatgrass, Kernza ® (K). Immediately adjacent to this agricultural field is a naturally occurring uncultivated area which has been studied as an ecological refugia (Duff et al., 2024), and was selected as the uncultivated area for this comparison. We sampled the upper portion of the coulee with minimal topographic difference compared to the paired cultivated site, but it did have differences in aspect. The uncultivated site adjacent to P-SW faced northeast, and the uncultivated site adjacent to K faced northwest.

To measure impacts of soil acidification on soil biological activity, we selected one field at the Fort Benton farm that had large bare spots amidst a stand of third-year alfalfa, due to low soil pH (Table 3.2). This field was cropped under a wheat-fallow rotation for around four decades, then seeded into alfalfa four years prior to this study. The bare spots selected for assessment were a minimum of 40 m diameter, but up to 140 m, in the 81 ha field. We employed a paired study design, where six bare, low pH, areas of the field were paired with six adjacent

and neutral pH zones with established fifth-year alfalfa to compare differences in soil health indicators in intensely cropped and acidified soils.

2.3 Sample Processing

Soils were sampled on May 16, 2022, and May 1, 2023, at Forsyth, and May 4, 2023, at Fort Benton. For each sampling location, seven soil cores (2.5cm dia x 15cm depth) were collected and composited from the area of the cropped field, the uncultivated area, and the acidified bare spots. In uncultivated areas with a shift in plant communities across the area, generally because of topographic relief, two separate areas were demarcated systematically and then sampled randomly, each with a distinct paired site from the cultivated soils. The soil corer was flame-sterilized after rinsing with 97% ethanol between each composited sample. The field-moist samples were then passed through a 2-mm sieve and stored at 4 °C for a maximum of 30 days prior to analysis. Soil textures were classified via Particle Size Analysis Hydrometer method (Gavlak et al., 2003). Soil pH was assessed with a 1:1 soil to water paste and measured with an Orion ROSS Ultra pH/ATC triode (USDA-NRCS, 2004).

2.4 Soil Analysis

Soil extracellular enzyme activity was measured in 1 g field-moist soils and incubated with a p-nitrophenol enzyme-specific substrate for 1 hour at 37 °C, then assayed for a colorimetric response. One control and two lab replicates were analyzed, following the procedure outlined by Dick et al. (1996) and Parham and Deng (2000). Five enzymes were analyzed: including β-1,4-N-acetyl glucosaminidase (EC 3.2.1.30, nitrogen cycling), β-1,4-glucosidase (EC 3.2.1.21, carbon cycling), arylsulfatase (EC 3.1.6.1, sulfur cycling), and acid and alkaline phosphatases (EC 3.1.3.1/2, phosphorus cycling). A proxy of total enzyme activity was

calculated via the geometric mean of the individual enzyme activities (García-Ruiz et al., 2008). If an individual enzyme yielded no activity, the zero was replaced with 0.01, half of the detection limit of enzyme activity; this allowed for approximation of the geometric mean with minimum activity from that specific enzyme.

Soil POxC was measured in duplicate samples of slurries comprised of 2.5 g of soil, 18 mL of deionized water, 2 mL of 0.2 M potassium permanganate ($KMnO_4$) in 1 M calcium chloride ($CaCl_2$), then shaken (2 min, 120 rpm) and settled (10 min, in darkness, at 25 °C) as outlined by Weil et al. (2003). The supernatant was diluted in deionized water (0.5 mL in 49.5 mL) and assessed for a colorimetric response.

PMN was analyzed in lab triplicates with a 14 d anerobic lab incubation at 30 °C, following methods developed by Keeney and Nelson (1982). Samples were assessed for NH_4^+ before and after incubation using a 1 M potassium chloride (KCl) extraction on a Lachat reduction analyzer (Lachat Instruments, Loveland, CO), with PMN calculated as the difference in NH_4^+ at time zero and 14 days.

Total C (TC) and TN were analyzed in 0.2 g of airdried and pulverized soil on a LECO combustion analyzer (LECO Corporation, St. Joseph, MI). Next, inorganic carbon (IC) was measured in all samples with a pH > 7.5 or C:N > 13 because soils with either criterion indicate important amounts of IC based on our previous work. The soils that met either criterion accounted for 54% of all soil samples. IC was measured via the modified-calcimeter method (Sherrod et al., 2002). Soils were acidified with 2 mL of 6 M HCl, sealed and rested, then the voltage was recorded on a pressure transducer and voltmeter at the peak of pressure. After

determination of SOC, SOM was calculated using a conversion factor of 1.72 (Khatoon et al., 2017).

2.5 Statistical Analysis

The R statistical package (v 4.1.2) was used for all statistical analyses in this study (R Core Team 2021). All data were assessed for the statistical assumptions of normality with a Shapiro-Wilk test (Wickham et al., 2023). Cook's distance with a limit four times the mean was utilized for assessing outliers (R Core Team 2021). Paired t-tests were used to test the effects of cultivation on biological soil health metrics, with pairs between cultivated and uncultivated soil samples and pairs between acid soils with neutral pH soils. Each paired sample was analyzed separately for individual years, due to potential differences in abiotic and management conditions from year to year. Further, a correlation matrix was created using Pearson's coefficient (r) to test for associations between SOM and all soil health indicators, including geometric mean of enzyme activity, five soil enzymes (ß-glucosidase, ß-glucosaminidase, acid and alkaline phosphatases, and arylsulfatase), total phosphatase, PMN, and POxC for uncultivated and cultivated soils, with an alpha level of 0.10 (Harrell, 2022).

3. Results

3.1 Soil Health Gap in Uncultivated and Cultivated Soils

For our first research objective, we predicted that cultivated soils would have lower soil enzyme activity compared to uncultivated soils. Across all paired samples and both study years, soil enzyme activity was lower with cultivation by 59%, on average, in the geometric mean of

enzyme activity in cultivated soils when compared to their uncultivated counterparts (Figure 3.1 A; mean of differences: 43.4 mg PNP·kg soil^{-1}·h^{-1}, $t_9 = 5.49$, $p < 0.001$).

The activity of individual enzymes also was lower in cultivated soils, across all paired samples (Table 3.3). Total phosphatase activity, the summation of acid and alkaline phosphatases, averaged 42% lower in cultivated soils than uncultivated soils (mean of differences: 143.1 mg PNP·kg soil^{-1}·h^{-1}, $t_9 = 4.94$, $p < 0.001$). β-glucosidase activity was 59% lower in cultivated soils than at uncultivated sites (mean of differences: 58.5 mg PNP·kg soil^{-1}·h^{-1}, $t_9 = 2.32$, $p < 0.05$). Arylsulfatase activity also was 59% lower on average in cultivated soils than uncultivated soils (mean of differences: 36.0 mg PNP·kg soil^{-1}·h^{-1}, $t_9 = 4.84$, $p < 0.001$). Lastly, β-glucosaminidase activity had the largest relative difference in cultivated soils amongst all enzymes tested, with 66% lower average activity in cultivated than uncultivated soils (mean of differences: 15.2 mg PNP·kg soil^{-1}·h^{-1}, $t_9 = 4.17$, $p < 0.01$).

We expected that cultivation would have lower PMN compared to uncultivated soils. PMN ranged from 3.8 to 22.4 mg N·kg soil^{-1} in the cultivated soils and 16.8 to 48.4 mg N·kg soil^{-1} in the uncultivated soils. Ultimately, PMN was 15.3 mg N·kg soil^{-1} lower on average in cultivated soils compared to their uncultivated counterpart (Figure 3.2 A; $t_9 = 3.46$, $p < 0.01$), accounting for a 51% reduction on average across all pairs.

We hypothesized that soil POxC would be lower in cultivated soils compared to adjacent uncultivated soils. POxC ranged from roughly 300 to 875 mg C·kg soil^{-1} in the cultivated soils, and from 460 to nearly 1300 mg C·kg soil^{-1} in the uncultivated soils, with cultivated soils having 39% less POxC on average than uncultivated soils. Specifically, the average difference in POxC from paired comparisons of uncultivated and cultivated soils was 338 mg C·kg soil^{-1} (Figure 3.3

A; $t_9 = 2.93$, $p < 0.05$). However, the two dryland paired samples from the Forsyth site in Year 2 of sampling had higher POxC under cultivated management conditions compared to the nearby uncultivated sites.

Soil organic matter was largely impacted by cultivation, with average SOM content ranging from 1.3 – 3.2% in cultivated soils and 1.8 – 6.4% in uncultivated soils. Generally, cultivated soils had 39% less SOM across all paired samples. This represents a difference of 1.3% SOM on average when comparing cultivated to uncultivated soils (Figure 3.3 A; $t_9 = 4.22$, $p < 0.01$).

3.2 Soil Health Gap across All Biological Parameters

Overall, the act of cultivation has serious implications on soil health traits, resulting in decreased soil health metrics by nearly 50% (Figure 3.5). When assessing all soil health traits in this study combined – soil extracellular enzyme activity, PMN, POxC, and SOM – soil health was 47% lower in cultivated soils than uncultivated soils.

3.3 Soil Biological Response to Acidification

We expected that all soil biological indicators would be lower in acidified soils compared to soils with established alfalfa and neutral pH, largely due to pH constraints on soil productivity. Instead, only enzyme activity decreased. The geometric mean of enzyme activity was lower in acidified soils compared to neutral pH soils by an average of 92.5% in overall enzyme activity across all paired sites (Table 3.4; mean of differences: 17.5 mg PNP·kg soil^{-1}·h^{-1}, $t_5 = 4.66$, $p < 0.01$). However, this result is heavily influenced by the fact that there was zero arylsulfatase activity in any of the acid soils, greatly reducing the measure of central tendency. When the geometric mean was calculated for four soil enzymes and excluding arylsulfatase, enzyme

activity was 42% lower in acid soils compared to neutral pH soils (Figure 3.1; mean of differences: 17.9 mg PNP·kg soil^{-1}·h^{-1}, $t_5 = 2.52$, $p < 0.05$). Alkaline phosphatase and ß-glucosidase were the only other enzymes that were lower in activity in acidified soils compared to adjacent neutral pH soils, though with less significance (Table 3.4; $p = 0.1$).

Other measures of soil biological activity did not differ between low pH soils and neutral pH soils within an alfalfa stand. PMN did not differ between acid and neutral pH soils across all paired sites (Figure 3.2 B; $t_5 = 0.74$, $p = 0.5$), and soil POxC did not differ (Figure 3.3 B; $t_5 = 0.64$, $p = 0.6$). However, there were significant differences in SOM, where neutral pH soils were 0.27% lower in SOM content compared to acidified soils across all paired sites (Figure 3.4 B; $t_5 = 3.43$, $p < 0.05$), representing 11.8% lower SOM on average in neutral pH soils.

4. Discussion

Indicators of soil biological activity were significantly lower in soils under crop production compared to adjacent uncultivated soils. Specifically, the geometric mean of soil extracellular enzyme activity was 59% lower, PMN was 51% lower, and both POxC and SOM were 39% lower, suggesting a soil health gap of 47% across all soil health parameters between cropped soils and uncultivated soils in these ten paired sampling sites. We found evidence that biological soil health is lower when soils are under crop production, but the degree of impact varied from field to field. Further, we discovered that soil acidification does not consistently contribute to less soil biological activity across all soil health parameters compared to adjacent cultivated soils without acidification issues, aside from enzyme activity.

4.1 Effects of Land Management on the Soil Health Gap

Other research on paired-site comparisons has also documented lower measures of soil health in cultivated soils compared to nearby grasslands. In the more humid and temperate soils of Siena, Italy, enzyme activities were 67-86% lower in continuous corn cultivated soils when compared to adjacent undisturbed grasslands (Saviozzi et al., 2001). Further, a study in the prairie pothole region of the NGP, in northern Iowa and southern Minnesota, found that cultivated soils had 92% less PMN than native grassland soils (De et al., 2020), however these sites were not paired comparisons. Within one year of converting a native grassland to wheat production in western South Dakota, there were significant decreases in POxC (Graham et al., 2021). This demonstrates the rapid response of POxC as a soil health indicator.

Most of the research on paired-site comparisons focuses on SOC, but the impacts from cultivation on SOC and SOM are not consistent or definitive, rather they respond to the intensity of the cultivation strategies. There is a significant decrease in soil function when grassland soils are cultivated, but there is also a gradient of soil health decline in cultivated systems, depending on the tillage, crop rotations, inclusion of cover crops, organic vs. conventional practices, and annual vs. perennial crops (Drexler et al., 2022; Maharjan et al., 2020; McLauchlan et al., 2006). In the central GP, no-till cultivated soils had 50% lower SOC than nearby grassland soils, and soils under conventional tillage were an additional 18% lower in SOC compared to the no-till soils (Maharjan et al., 2020). Incorporation of cereals and high residue crops in rotations outside of the NGP lessens the extent of SOC decline, while high proportions of root crops, like sugar beet, in rotations in Germany have greater impacts on SOC decline (Capriel, 2013). Further, incorporation of perennial grains into crop rotations resulted in increased SOM; these effects were seen in semi-arid cropping systems in Alberta after a two-year time period (Kim et al.,

2022). This is likely because SOC in cropping systems is driven by the quantity of OM inputs, both aboveground and belowground, and their decomposition rate, ultimately impacting the ability to build SOM (Gurmu, 2020). The total decline in SOM can be mitigated by management practices that increase residue inputs while minimizing soil disturbance.

Livestock grazing on rangelands and grasslands can also have impacts on SOC stocks, SOM accrual capacity, soil structure, and some soil health indicators (Abdalla et al., 2018). The degree of impact on SOM varies based on both the timing and intensity of grazing, because of its impact on litter accumulation and decomposition (Naeth et al., 1991). Heavy intensity and early season grazing on Canadian NGP grasslands is associated with decreased SOM (Naeth et al., 1991). However, in NGP grasslands with minimal grazing intensity, SOC stocks tend to be more stable over decadal timescales, regardless of weather and climate patterns (Ingram et al., 2008). Most of the uncultivated sites selected for this study faced a very minimal grazing intensity, with two uncultivated sites, near the upland dryland fields in Forsyth, experiencing more moderate grazing pressure (C. Steiger, personal communication, March 22, 2024).

4.2 The SOM Gap as a Predictor of the Soil Health Gap

Management of SOM is vital for both the maintenance and improvement of soil health (Lal, 2016). Native grassland soils have higher proportions of SOM than soils under crop production, often reaching a 50% decline of SOM in cultivated soils in the NGP, likely because of the removal of plant materials at harvest (Jones et al., 2002; Mann, 1986). Our results are within the range of previously documented SOC and SOM declines in the cultivated NGP soils of 42 ± 11% (Haas & Evans, 1957; Liebig et al., 2006). It is estimated that soils converted back to grasslands after cultivation will reach similar SOM pools as their uncultivated counterparts in

50-75 years (McLauchlan et al., 2006), representing the temporal effects of this decline. While the fraction of SOM in soils is relatively small by weight, it provisions a disproportionately large amount of ecological processes within a soil, including driving its biological activity by providing some of the energy and constituent materials for soil microorganisms (Gurmu, 2020). SOM may be the single best integrator of soil productivity, as it has major impacts on the biologically active portion of soils (Wander et al., 1996). Further, we have previously documented a strong positive correlation between SOM in soils and the biological indicator of extracellular enzyme activity (Chapter 2).

At the Forsyth site, the smallest numerical difference in biological soil indicators between cropped and uncultivated soils was from a dryland field under a P-WW rotation, where the uncultivated soils were moderately grazed compared to our other uncultivated sites, which had minimal grazing impact. Further, this cultivated site had a very high amount of crop residue on the soil surface. Specifically, this paired system only had a 3.1 mg PNP·kg soil^{-1}·h^{1} difference in the geometric mean of enzyme activity (Table 3.2), representing an 8.7% lower activity, a smaller difference than measured at all other paired sites. In fact, the activities of three individual enzymes, acid phosphatase, β-glucosaminidase, and β-glucosidase, as well as concentrations of soil POxC, were greater in the cropped soil than in the uncultivated soil as well. This may be explained by the similar SOM content between these paired cropped and adjacent grassland soils, with SOM percentages of 1.7% and 1.8% respectively. All other paired cultivated and uncultivated sites showed greater differences in enzyme activity from cropped soils to uncultivated soils, however these pairs also had a larger gap in SOM. The gap in SOM can help explain and predict the gap in soil biological health indicators, like enzyme activity, between

these paired uncultivated and cultivated sites. This link between SOM and biological activity emphasizes the importance of prioritizing SOM management to increase overall soil health, and ultimately close the soil health gap between cropped soils and native grassland ecosystems.

There was a strong positive correlation between SOM and soil biological indicators across almost all indicators assessed in this study, including the geometric mean of enzyme activity, ß-glucosidase, ß-glucosaminidase, arylsulfatase, alkaline phosphatase, total phosphatase, PMN, and POXC (Table 3.5). This provides an opportunity for a using a simplistic approach, by only measuring SOM, for estimating a soil's functional capacity for nutrient cycling, plant available N, and C dynamics. Further, evaluation of SOM is already documented as a prominent indicator of soil health and is widely-adopted and understood by producers (Obalum et al., 2017; Stott, 2019). The accessibility of SOM as a soil health indicator also makes it a plausible candidate for widespread use, especially for minimizing the time and cost to determine the soil health gap and set realistic and site-specific soil health benchmarks of the maximum attainable capacity of a soil within a specific agroecosystem.

However, SOM is not as sensitive of a soil health indicator to change as other biological parameters and is slow to respond to change. While SOM acts as a driver of many biological functions, it does not respond to change quickly, often taking over five years to change (Lehman et al., 2015b). Extracellular enzyme activity, PMN, and POxC are all more sensitive to change, even from year-to-year, capturing the biological response to both potential changes in weather conditions (abiotic factors) and crops grown (biotic factors). Specifically, SOM and POxC were both lower by 39% in cultivated sites, but POxC was much more variable than the stable SOM parameter. Assessing more sensitive soil health parameters in agricultural lands can provide an

earlier indication of the direction of change in soil health, allowing for a more fine-tuned management approach on a shorter timescale.

4.3 Confounding Effects in Acidified Soils

Acidification is a factor of intensive crop production, over fertilization of N (specifically in ammoniacal form), and poor N use efficiency (Yadav et al., 2020). We had hypothesized that soils experiencing acidification would have lower soil biological activity than areas with an established healthy stand of fifth-year alfalfa with neutral pH. We expected that acid soils would be associated with lower levels of SOM, primarily because bare soils without plant cover have no source of organic inputs. However, we discovered that acidic soils had slightly greater SOM compared to neutral pH soils, with 2.0% SOM in neutral pH soils and 2.3% SOM in acidic soils, accounting for 11.8% lower SOM on average in the neutral pH soils. It is possible that a lower pH may depress SOM decomposition by decreasing microbial activity, thus increasing protection of SOC by mineral phases. The higher SOM in acidic soils could also be caused by their topographic locations in the field, where acidic soils were dominantly found in lower, wetter areas of the field that likely had higher rates of nitrate leaching leading to the acidification. These areas also could have had higher SOM to begin with because of greater moisture availability. These findings of high SOM in acid soils are opposite of the relationship observed between SOC and pH in the semi-arid climate of the Columbia Basin, Oregon in long-term winter wheat-summer fallow rotations, where SOC decreased with decreasing pH in the 0 – 20 cm depth, regardless of N fertilizer rates, even at pH values below 5.0 (Ghimire et al., 2017). This indicates that SOM may not be a reasonable or consistent predictor of soil biological function in acidified soils.

Acidified soils showed a completely suppressed response in arylsulfatase activity. This is consistent with other findings on the effects of continuous N fertilizer and the consequent soil acidification on arylsulfatase activity, though this research was in a humid tropical forest ecosystem (Chen et al., 2016; Mori et al., 2020). Other studies in semi-arid agricultural systems with long-term winter wheat-summer fallow rotations in eastern Oregon documented significantly decreased arylsulfatase activities in the high N fertilizer (urea) treatments with soil pH around 4.8, with an average of 2 mg PNP·kg soil^{-1}·h^{-1} of arylsulfatase activity, compared to 6-10 mg PNP·kg soil^{-1}·h^{-1} in the no N and manure-N treatments which also had higher soil pH of 5.7-6.7 (Reardon et al., 2022). This consistent pattern between arylsulfatase activity and soil pH could indicate that acidification may slow down S cycling by inhibiting S mineralization in acidified soils (Chen et al., 2016). The optimal pH of arylsulfatase activity is at a remarkably low pH of 3, which is lower than all other enzymes (Turner, 2010), indicating that the completely diminished activity observed in this study is not due to inability of the enzyme to bind at low pH in laboratory incubations. Therefore, it is possible that the acidic soils provided an ideal condition, where a lower amount of arylsulfatase production could catalyze enough S production to meet the demand (Mori et al., 2020), resulting in an activity not observed above the detection limit or an activity that has ceased due to sufficient available S.

After removing arylsulfatase activity from the measure of total enzyme activity, and calculating the geometric mean based on the activities of ß-glucosidase, ß-glucosaminidase, and acid and alkaline phosphatases, the geometric mean of enzyme activity was 42% lower in acidified soils compared to neutral pH soils; this is comparable to the difference observed between uncultivated and cultivated soils. In fact, soil enzyme activity is the sole parameter that

captured a difference in the biological function of acidified soils. This relationship suggests that enzyme activity is a particularly sensitive indicator of soil biological health, at least with respect to acidity, and is a valuable parameter to assess the direction of change in soil health within cultivated soils. Assessment of total extracellular enzyme activity, or even arylsulfatase alone, may discern issues with acidification, or indication of decline, prior to any other soil health measures.

4.4 Implications & Future Research Needs

Uncultivated soils can provide a baseline reference of the potential maximum function and optimized health of a soil if it had never transitioned into crop production. While this benchmark is an unrealistic standard to compare agricultural soils to, it offers a glimpse into the relative difference of a soil's biological activity, and ultimately a vision for how agricultural lands may be optimized for continued productivity and sustainability. Alternative agricultural systems that closely follow the biodiversity, phenology, and biogeochemical cycles of native grassland ecosystems are key for protecting soil resources for future generations (DeLuca & Zabinski, 2011). This case study was conducted on seven fields and their adjacent grasslands across two farms in Montana, but the results are consistent with other research comparing SOM and soil health response in cultivated soils and across fence lines on uncultivated soils in the NGP (Jones et al., 2002; Kaye et al., 2005; Liang et al., 2023; Malo et al., 2005; Mann, 1986). We did not find consistent differences in soil biological health parameters between acid and neutral pH soils, but the enzymatic difference was substantial. Therefore, future experiments that are conducted on a wider range of climate regimes and across variations of agricultural management practices may reveal more relevant insights on the biological soil health gap,

whether the SOM gap presents a realistic benchmark approach, and the response to acidification in other climate regimes.

Table 3.1 Soil characteristics for a 0 – 15 cm depth, based on composited samples collected in Forsyth on May 16, 2022, and May 1, 2023, and Fort Benton on May 4, 2023, plus the cultivated soil's prior two-year crop sequence for Fort Benton cultivated (Ag) and uncultivated (Unc) paired sites. The lowercase letter following the crop sequence acronym denotes whether the field was irrigated (i) or dryland (d).

Forsyth, MT																
Sequence*	M-FB(i)		L-S(d)		WW-SW(d)		FB-SB(i)		FB-SB(i)		S-P(d)		SW-WW(d)		P-WW(d)	
Paired Site	Ag	Unc	Ag	Unc	Ag	Unc	Ag	Unc	Ag	Unc	Ag	Unc	Ag	Unc	Ag	Unc
Texture**	SL	SL	L	L	SL	SL	L	L	L	SL	SiL	SiCL	SL	SL	L	SL
Clay (%)	17.6	13.4	17.8	21.7	15.5	10.0	20.8	15.5	20.1	12.0	21.0	34.0	16.4	17.6	17.2	13.3
pH	8.0	7.9	8.2	7.8	6.1	7.1	8.0	7.9	7.7	7.3	8.2	7.8	6.1	7.1	6.1	7.8
SOM (%)	2.3	3.7	1.9	3.5	1.3	2.0	2.3	3.7	2.3	3.7	1.9	3.5	1.3	2.0	1.7	1.8
TC (%)	2.1	3.2	1.8	3.2	1.0	1.2	2.2	2.4	2.0	2.2	1.7	2.7	0.8	1.2	1.0	1.1
TN (%)	0.10	0.26	0.13	0.23	0.10	0.12	0.11	0.16	0.11	0.15	0.08	0.12	0.06	0.07	0.06	0.06

*Crop sequences: M-FB (millet-faba bean), L-S (lentil-sunflower), WW-SW (winter wheat-spring wheat), FB-SB (faba bean-sugar beet), S-P (sunflower-pea), SW-WW (spring wheat-winter wheat), P-WW (pea-winter wheat). ** Texture: sandy (S), loam (L), silty (Si), clay (C).

Fort Benton, MT				
Sequence*	P-SW(d)		K(d)	
Paired Site	Ag	Unc	Ag	Unc
Texture**	L	L	L	L
Clay (%)	26.1	21.7	22.0	26.9
pH	7.6	7.5	7.9	7.8
SOM (%)	2.6	6.4	3.2	3.9
TC (%)	1.6	3.8	1.9	3.3
TN (%)	0.14	0.36	0.21	0.25

*Crop sequences: P-SW (pea-spring wheat), K (intermediate wheatgrass Kernza). **Texture: loam (L).

Table 3.2 Soil characteristics for a 0 – 15 cm depth, based on composited samples collected the Fort Benton neural pH alfalfa (Alf) and acidified bare (Bare) paired sites.

	Alf-B-1		Alf-B-2		Alf-B-3		Alf-B-4		Alf-B-5		Alf-B-6	
Paired Site	Alf	Bare	Alf	Bare	Alf	Bare	Alf	Bare	Alf	Bare	Alf	Bare
Texture*	SCL	L	CL	L	SCL	L	L	L	L	L	L	L
Clay (%)	25.5	20.5	27.0	15.6	22.6	21.5	23.9	20.8	21.7	19.6	22.6	19.0
pH	7.2	5.8	6.3	4.5	5.0	4.5	5.8	4.7	7.8	4.6	7.9	4.6
SOM (%)	2.0	2.3	2.2	2.2	2.1	2.4	2.0	2.2	1.8	2.4	1.9	2.3
TC (%)	1.2	1.4	1.3	1.3	1.2	1.4	1.2	1.3	1.3	1.4	1.5	1.3
TN (%)	0.11	0.11	0.11	0.11	0.13	0.11	0.11	0.11	0.11	0.12	0.10	0.12

*Texture acronyms: sandy (S), clay (C), loam (L)

Table 3.3 Enzymatic activity (mg PNP g soil^{-1} hr^{-1}) of five soil enzymes (ß-glucosidase, ß-glucosaminidase, acid and alkaline phosphatases, and arylsulfatase), total phosphatase, and the geometric mean of all enzyme activities for all paired cultivated (Ag) and uncultivated (Unc) soils.

	Acid Phosphatase		Alkaline Phosphatase		Total Phosphatase		Arylsulfatase		ß-glucosidase		ß-glucosaminidase		Geometric mean	
Paired Site	Ag	Unc	Ag	Unc	Ag	Unc	Ag	Unc	Ag	Unc	Ag	Unc	Ag	Unc
M-FB(i)	13.8	96.3	145.6	317.3	159.5	413.7	32.0	99.4	27.5	283.0	13.7	35.2	30.0	124.8
L-S(d)	13.4	66.1	198.3	296.4	211.7	362.5	33.1	71.9	55.8	94.1	10.4	27.6	34.8	81.8
WW-SW(d)	146.1	164.9	23.8	107.3	169.9	272.2	13.9	27.1	19.4	60.1	8.3	18.9	23.9	55.9
FB-SB-1(i)	39.3	77.7	166.0	394.3	205.3	472.0	40.3	81.5	40.3	122.0	6.2	22.1	36.6	92.4
FB-SB-2(i)	35.5	191.1	161.6	250.6	197.1	441.7	33.4	32.8	42.4	50.8	1.2	28.7	24.9	74.5
S-P(d)	22.1	45.5	155.9	237.8	178.0	283.3	28.4	58.9	36.7	51.9	0.8	15.9	19.7	55.5
SW-WW(d)	117.2	165.9	26.5	107.2	143.6	273.1	6.6	33.7	7.5	43.5	4.3	14.3	14.6	51.8
P-WW(d)	120.5	106.6	38.7	67.6	159.2	174.2	14.1	29.1	35.1	20.8	14.4	12.1	31.9	35.0
P-SW(d)	191.4	182.9	126.4	145.3	317.8	328.2	13.3	78.8	49.8	170.3	6.5	42.8	34.9	95.1
K(d)	85.7	112.0	123.8	249.3	209.5	361.3	36.1	98.1	94.8	98.1	11.7	12.5	51.0	69.3
p-value	<0.05		<0.01		<0.01		<0.01		<0.05		<0.01		<0.01	
$\lvert t\text{-stat}_9 \rvert$	2.74		5.11		4.94		4.84		2.32		4.17		5.49	
Mean of Differences	42.4		100.6		143.1		36.0		58.5		15.2		43.4	

Table 3.4 Enzymatic activity (mg PNP g soil^{-1} hr^{-1}) of five soil enzymes (ß-glucosidase, ß-glucosaminidase, acid and alkaline phosphatases, and arylsulfatase), total phosphatase, and the geometric mean of all enzyme activities, with and without arylsulfatase, for all paired neutral pH alfalfa (Alf) and acidified bare (Bare) soils in Ft. Benton. Values of 0 were replaced with 0.01, half the detection limit, in geometric mean calculations.

	Acid Phosphatase		Alkaline Phosphatase		Total Phosphatase		Arylsulfatase		ß-glucosidase		ß-glucosaminidase		Geometric mean		Geom Mean w/o Aryl	
Paired Site	Alf	Bare	Alf	Bare	Alf	Bare	Alf	Bare	Alf	Bare	Alf	Bare	Alf	Bare	Alf	Bare
Alf-B-1	121.6	137.6	27.3	25.0	148.9	162.6	5.7	0	26.2	19.1	15.1	6.0	22.9	2.2	45.5	36.4
Alf-B-2	181.9	69.0	35.1	0	217.0	69.0	7.9	0	53.5	12.1	5.8	4.8	26.0	0.1	53.3	4.9
Alf-B-3	117.6	132.5	15.3	24.5	132.8	157.0	0	0	20.3	18.3	4.8	4.1	1.1	1.8	29.7	32.8
Alf-B-4	140.3	119.0	26.4	12.9	166.8	131.9	6.3	0	54.5	17.4	6.7	4.1	21.5	1.5	46.8	27.0
Alf-B-5	74.8	122.3	102.9	3.9	177.8	126.2	18.3	0	32.3	34.4	1.8	7.1	20.8	1.6	37.9	27.0
Alf-B-6	79.6	97.9	153.3	2.4	232.9	100.2	21.8	0	38.4	25.8	1.4	6.2	21.1	1.3	43.1	20.6
p-value	0.8		0.1		0.1		<0.05		0.1		0.8		<0.01		<0.05	
$\lvert t\text{-stat}_5 \rvert$	0.27		1.89		1.86		2.96		2.17		0.25		4.66		2.52	
Mean of Diff	6.26		48.6		54.9		10.0		16.3		0.55		17.5		17.9	

Table 3.5 Pearson correlations (r) between SOM and the geometric mean of enzyme activity, five soil enzymes (ß-glucosidase, ß-glucosaminidase, acid and alkaline phosphatases, and arylsulfatase), total phosphatase, PMN, and POXC for uncultivated and cultivated soils.

Soil Biological Response	r
Acid Phosphatase	0.22
Alkaline Phosphatase	0.57*
Total Phosphatase	0.69**
Arylsulfatase	0.77**
ß-glucosidase	0.69**
ß-glucosaminidase	0.79**
Geometric Mean	0.79**
PMN	0.82**
POXC	0.75**

p-value ** <0.001; * <0.01

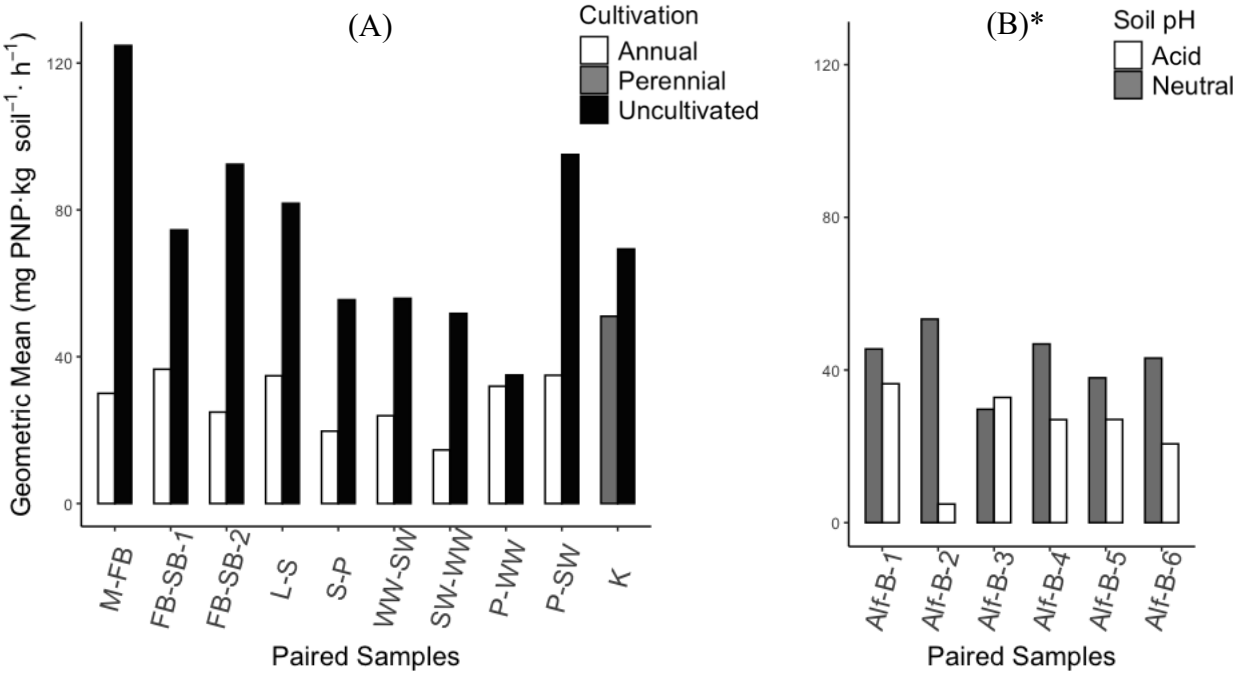

Figure 3.1 Geometric mean of enzyme activity (mg PNP·kg soil^{-1}·h^{-1}) for paired samples, represented by cultivation type in the comparison of uncultivated and cultivated soils in A (mean of differences: 43.4 mg PNP·kg soil^{-1}·h^{-1}, $t_5 = 5.49$, $p < 0.001$) and by soil pH in the comparison of acidified and neutral pH soils in B* (mean of differences: 17.9 mg PNP·kg soil^{-1}·h^{-1}, $t_5 = 2.52$, $p < 0.05$). *Note that the geometric mean calculation in B excludes arylsulfatase. Crop sequences: M-FB (millet-faba bean), L-S (lentil-sunflower), WW-SW (winter wheat-spring wheat), FB-SB (faba bean-sugar beet), S-P (sunflower-pea), SW-WW (spring wheat-winter wheat), P-WW (pea-winter wheat).

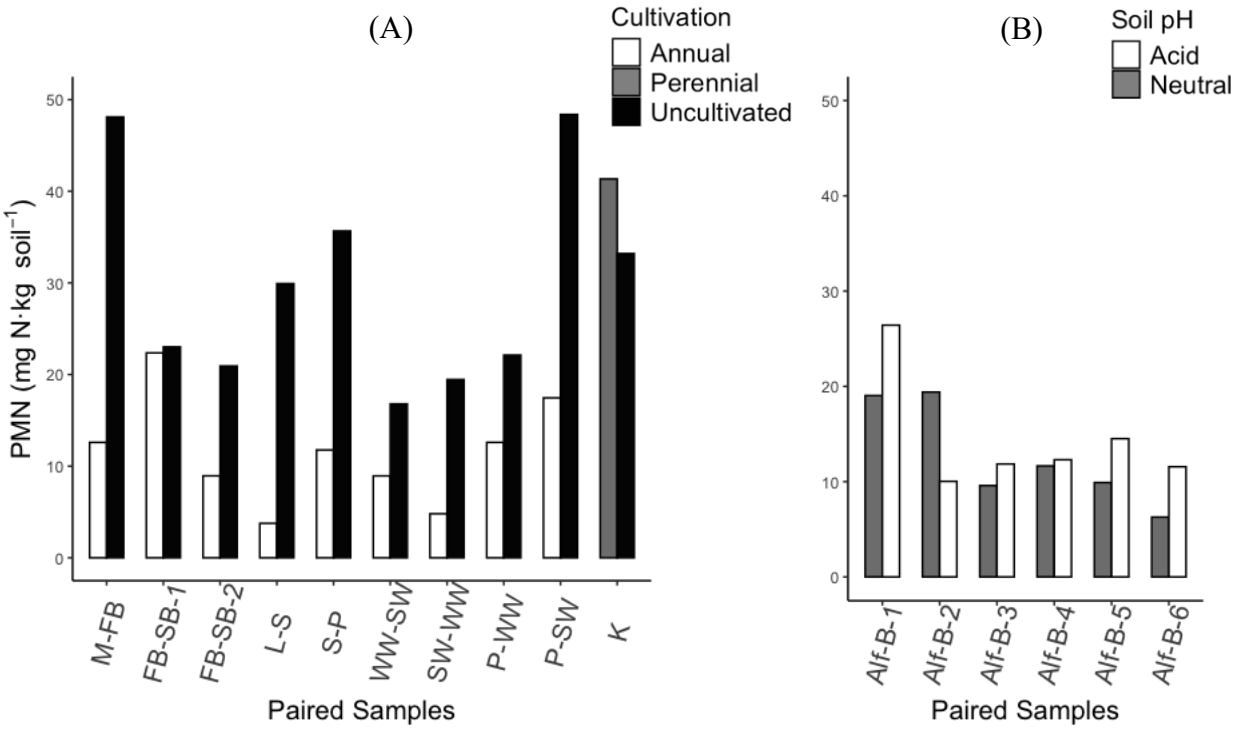

Figure 3.2 Potentially mineralizable nitrogen (mg N·kg soil^{-1}) for paired samples, represented by cultivation type in the comparison of uncultivated and cultivated soils in A (mean of differences: 15.3 mg N·kg soil^{-1}, $t_5 = 3.46$, $p < 0.01$) and by soil pH in the comparison of acidified and neutral pH soils in B (mean of differences: 0.27 mg N·kg soil^{-1}, $t_5 = 0.74$, $p = 0.5$). Crop sequences: M-FB (millet-faba bean), L-S (lentil-sunflower), WW-SW (winter wheat-spring wheat), FB-SB (faba bean-sugar beet), S-P (sunflower-pea), SW-WW (spring wheat-winter wheat), P-WW (pea-winter wheat).

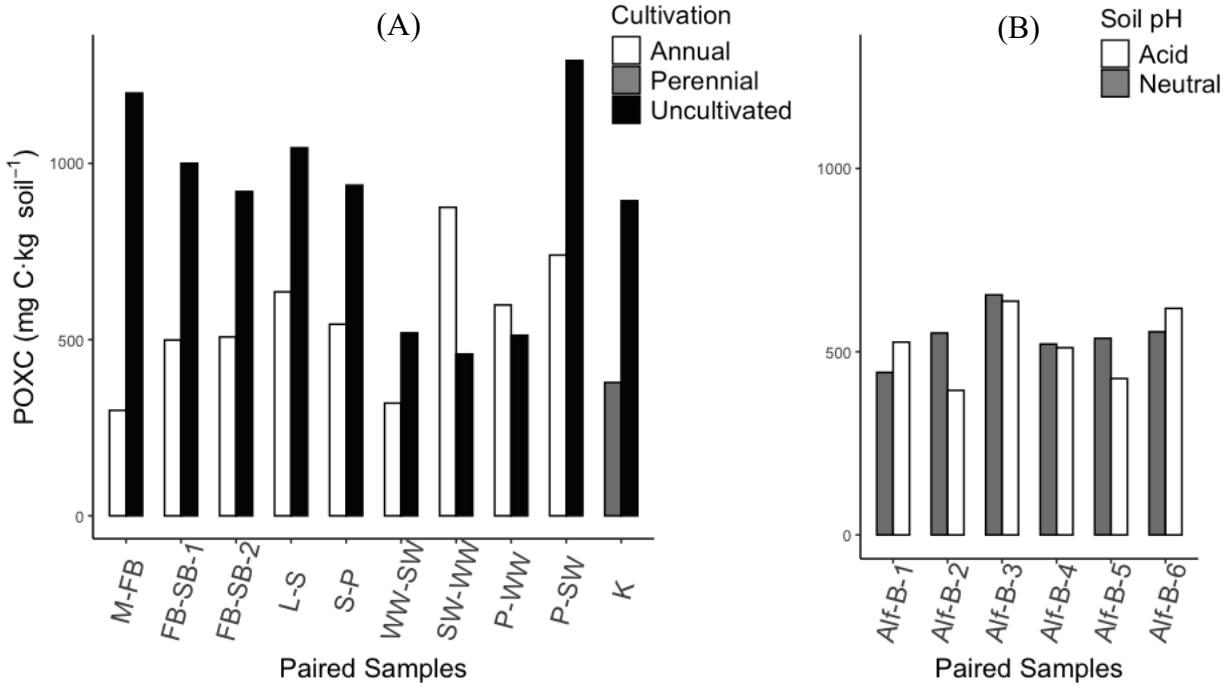

Figure 3.3 Permanganate oxidizable carbon (mg C·kg soil^{-1}) for paired samples, represented by cultivation type in the comparison of uncultivated and cultivated soils in A (mean of differences: 338.2 mg C·kg soil^{-1}, $t_5 = 2.93$, $p < 0.05$) and by soil pH in the comparison of acidified and neutral pH soils in B (mean of differences: 24.4 mg C·kg soil^{-1}, $t_5 = 0.64$, $p = 0.5$). Crop sequences in A: M-FB (millet-faba bean), L-S (lentil-sunflower), WW-SW (winter wheat-spring wheat), FB-SB (faba bean-sugar beet), S-P (sunflower-pea), SW-WW (spring wheat-winter wheat), P-WW (pea-winter wheat).

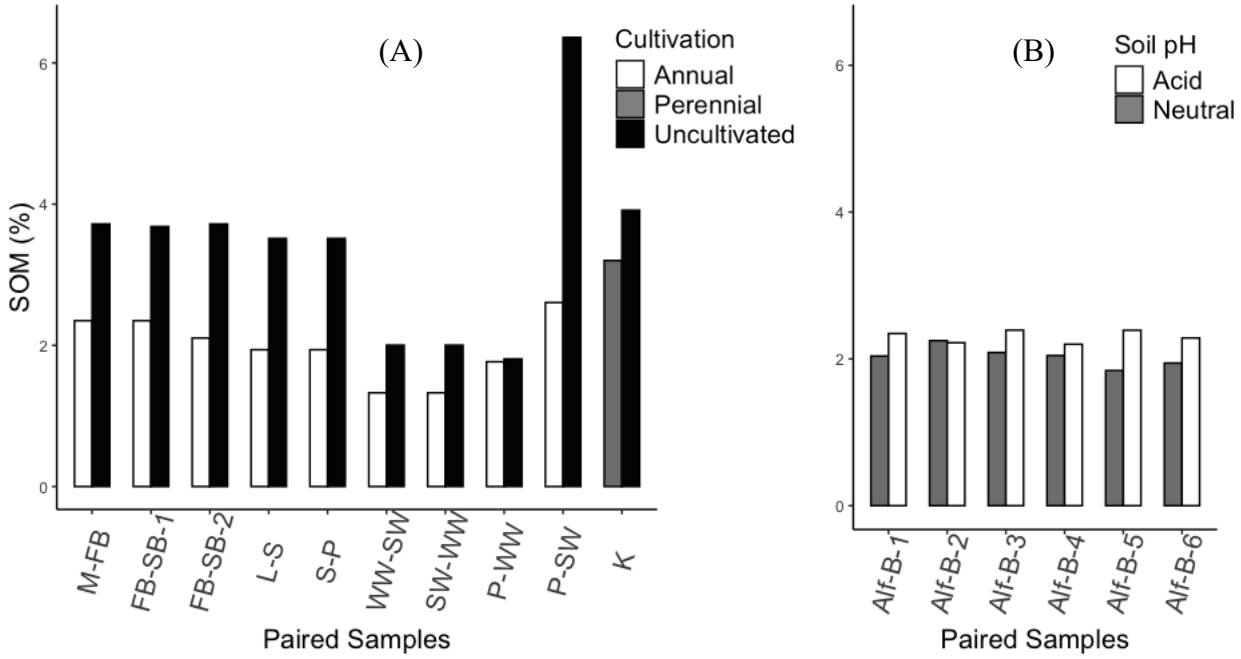

Figure 3.4 Soil organic matter content (%) for paired samples, represented by cultivation type in the comparison of uncultivated and cultivated soils in A (mean of differences: 1.3%, $t_5 = 4.22$, $p < 0.01$) and by soil pH in the comparison of acidified and neutral pH soils in B (mean of differences: 0.27%, $t_5 = 3.43$, $p < 0.05$). Crop sequences: M-FB (millet-faba bean), L-S (lentil-sunflower), WW-SW (winter wheat-spring wheat), FB-SB (faba bean-sugar beet), S-P (sunflower-pea), SW-WW (spring wheat-winter wheat), P-WW (pea-winter wheat).

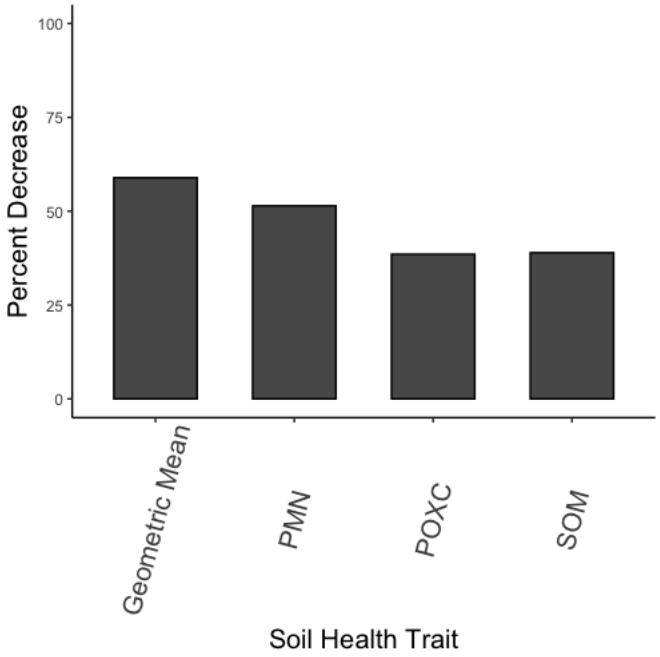

Figure 3.5 Percent decrease for all soil health traits – geometric mean of five enzymatic activities, potentially mineralizable nitrogen (PMN), permanganate oxidizable carbon (POXC), and soil organic matter (SOM) – between cultivated soils and uncultivated soils, not including acid and neutral pH pairs.

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CHAPTER FOUR

CONCLUSION

My research explored how soil biological indicators respond to cultivation in diverse cropping systems by comparing soil health parameters after different crop types in diverse crop sequences, and the degree to which these parameters decline compared to their uncultivated counterparts. The goal was to assess impacts to soil biological activity from cropping systems and quantify how soil health responds to cultivation generally. In semi-arid Montana, where wheat is the dominant crop grown, often either in wheat-fallow or continuous wheat systems, I wanted to assess soil health in diverse continuous-production cropping systems that rarely fallow and include multiple crops. This type of cropping system, which increases environmental sustainability and diversifies economic opportunities for farmers, is the realistic vision of sustainable agricultural production in the future. Crop production intensification is only one means to meeting the growing demands on our agricultural systems. The other option, though less favorable, is the conversion of more intact ecosystems into crop production (Tilman et al., 2011). In the NGP, native grasslands are the likely candidates for this type of land use change, though these ecosystems provision many ecosystem services that cannot be replicated in cultivated systems (Smart et al., 2021). Thus, I wanted to measure the change in soil health associated with cultivation of lands that were once native grasslands by direct comparisons of cultivated and uncultivated soils.

In Chapter 2, I showed that root crops, namely sugar beet, may drive the N mineralization potential associated with soilN, resulting in more plant available nitrogen following sugar beets. This pattern is likely linked to the crop residues returned to the soil during sugar beet harvest.

Sugar beet shoots are of high quality, i.e., low C:N, composed of 4-6% N with a C:N around 11 during the growing season (Varga et al., 2022), but falling to around 1.5% N with a C:N around 30 at senescence (Eslami et al., 1988). These residues can decompose rapidly, supplying the soil with ample mineral N. The low C:N of these crop residues reduces the risk of N immobilization by the decomposer community that often occurs with higher C:N residues—where residues slowly decompose and the increase in C causes the available N to be taken up primarily by soil biota to sustain activity and maintain microbial biomass, leaving little N available for crops (Jansson & Persson, 1982). I found greater PMN following sugar beets in two separate fields in this study.

Total extracellular enzyme activity at the beginning of the plant growing season, assessed via the geometric mean of five soil enzymes, did not follow a pattern based on the crop that it followed. However, results described in Chapters 2 and 3 revealed a pattern of enzyme activity that was strongly correlated with SOM. Ultimately, soils with high SOM had higher total enzyme activities. This phenomena may be explained by the additional resources in SOM that boost microbial activity and increased surface area of SOM, which provides ample opportunity for extracellular enzymes to bind to surfaces, becoming protected and stabilized within the soil matrix (Burns et al., 2013; Wallenstein & Weintraub, 2008). While this discovery may prompt assumptions that the more simplistic assessment of SOM could glean information about a soil's nutrient cycling capacity, SOM responds slowly to change. Soils may need up to five years to show accumulation of SOM in response to land management practices (Blanco & Lal, 2023; Lehman et al., 2015), whereas soil enzymes can detect and react to changes much more quickly. Soil enzyme activity could allow farmers to see the impacts of their crop rotations, tillage

practices, and even fertilizer management within a single growing season (Acosta-Martinez et al., 2011), and fine-tune their management approach year-to-year based on their soil health goals.

Soil POxC was the only soil health parameter that was not consistently lower in dryland systems compared to the more productive irrigated systems assessed in Chapter 2. While POxC was influenced by changes from year to year within the same field in Chapter 2, it is not clear what is driving the labile C responses in this study. However, in Chapter 3, POxC was the only biological indicator that was higher in some instances of cultivation compared to uncultivated sites. The two uncultivated sites that showed decreased POxC compared to cultivated soils were heavily grazed, and the cultivated sites had high quantities of overwintered crop residues, meaning that the timing and intensity of grazing could have major effects on labile C dynamics, and/or that overwintered residues could affect POxC.

Chapter 3 revealed a significant soil health gap between cultivated soils under long-term crop production and adjacent uncultivated soils. In this paired study design, cultivated soils were associated with lower soil enzyme activities, lower PMN, and lower POxC, often with reductions by nearly half in all of these soil biological health indicators. Overall, soil health across all parameters tested was 45% lower in cultivated systems than their uncultivated counterparts. This finding could have major implications on the overall perceived sustainability of cropping systems. These soil health indicators followed a similar decline as SOM itself, which could provide a simple and economically reasonable method for estimating the soil health gap within a specific agroecosystem. With an understanding of the gap in soil health between cropping systems and uncultivated land relative to a specific soil and place, farmers can identify the maximum attainable goal regarding their soils optimal function and productivity.

Generally, acidified soils (pH < 5) had lower soil enzyme activity than their paired adjacent neutral pH soils. The sensitivity of soil enzymes was highlighted in this paired comparison in Chapter 3, as they were the only soil health parameter that showed a consistently lower response in acid soils, with a geometric mean of total enzyme activity 93% lower in acid soils than neutral pH soils. This measure was exacerbated by one enzyme, arylsulfatase, which showed a completely diminished response in acidified soils with activity below the detection limit. However, even without arylsulfatase, enzymatic activity was still 44% lower in acidified soils compared to neutral pH soils. Both PMN and soil POxC did not decline in acidified soils in this study, demonstrating that some aspects of soil health, namely N availability and labile C, did not differ in soils with acid-induced productivity issues. Further, SOM was higher in acid soils compared to nearby neutral pH soils, indicating that SOM decomposition may be depressed at low pH due to microbial activity constraints, or that soils likely to decline in pH are also higher in SOM because of lower topography, more moist conditions, or even less lime initially.

Considerations for Future Research

Building from what I've learned through Chapter 2 of this study in the assessment of diverse crop sequences and their impacts on soil health, it would be beneficial to incorporate a more standardized crop rotation schema over a longer study period with participating farmers. This on-farm research did not provide us with an opportunity to manipulate crop sequences. However, it did allow for a realistic assessment of soil biological response in sequences used by farmers in the semi-arid NGP. For all soil health parameters aside from PMN, there were no patterns based on crop type that persisted across both study years regardless of field or other soil characteristics. If crops can impact soil health parameters within a single growing season, a more

controlled experiment would increase the likelihood that we could measure that effect, by designing an experiment with entire crop sequences over a longer timeframe. This temporal constraint limited our ability to assess the entire sequence, which may have offered more insight on potentially traceable impacts associated with crop types and the ability to make definitive statements about the impacts of crop sequences on soil biological activity.

In this study, we measured crop residues that overwintered and persisted to the beginning of the following growing season to assess relationships between soil health and persistent residues. This presented a challenge, where we were unable to estimate the proportion of crop residue which stayed on the soil surface, versus the proportion that had decomposed or was lost to wind or water transport. Because PMN was linked to a crop type, sugar beets, that had minimal persisting residues, we were unable to ascertain if PMN activity was responding to overwintered crop residues because the residues likely decomposed relatively quickly. Future research that measures crop residues both after harvest, and in the spring, at the beginning of the growing season, could address a relationship between decomposing residue and nutrient returns to soil, which may further explain the relationship observed in this study between sugar beet and PMN.

For Chapter 3, uncultivated site selection was informed by the observed biodiversity of a site and the farmer's memory of the history of that specific land. While the sites sampled in this study were never cultivated, some of them do experience periods of intensive grazing. For future research on paired-site comparisons, it may be beneficial to assess grazing intensity of uncultivated sites, and only select sites with more minimal grazing pressure to avoid highly perturbed grasslands in this assessment. An unknown history of disturbance via grazing could

diminish the differences observed in paired cultivated and uncultivated studies, leading to a true soil health gap between crop production and native grasslands that may be much larger. This research offers a starting point for paired comparisons of the impacts of cultivation on soil health in a semi-arid agroecosystem. Future research could incorporate a much broader array of paired cultivated and uncultivated lands, and in different climate regions, to assess how the soil health gap responds in different ecosystems and under different agricultural management practices. The patterns of decline may be more substantial than we reported in true intact grassland ecosystems without heavy grazing pressures and may also vary in regions with different climate and precipitation patterns.

I also conducted a case study on one field that was experiencing issues with soil acidification by comparing acid and neutral pH soil health. This work provides a baseline for assessing the impacts of soil acidification on soil biological activity and would benefit from other paired-site comparisons in differing cropping systems and climates. Paired-site comparisons offer a unique approach to understanding the impacts of soil acidification within a single field, and continued research could explain how this trend compares across different fields, farms, management practices, and climates.

Ultimately, soils are highly heterogenous systems, often making it difficult to collect enough data to represent the entire dynamic entity of agricultural soils or make reasonable conclusions that could be widely applicable to other cropping systems or in other regions. Continued research on soil health could help farmers to make decisions about cropping systems that maintain high productivity while increasing sustainability. This work contributed to the understanding of soil health in diverse cropping systems of semi-arid Montana and found

significant soil health gaps between cultivated and uncultivated systems. By integrating a conservation mindset with intensive continuous-crop production in agricultural landscapes simultaneously, farmers can make appreciable impacts to their soil health while maintaining high levels of productivity.

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