DISSERTATION

GROWING DEEPER: PATHWAYS TO ENHANCING SOIL ORGANIC MATTER IN ANNUAL AND PERENNIAL DRYLAND GRAIN AGROECOSYSTEMS

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ABSTRACT

GROWING DEEPER: PATHWAYS TO ENHANCING SOIL ORGANIC MATTER IN ANNUAL AND PERENNIAL DRYLAND GRAIN AGROECOSYSTEMS

The story of agriculture and human civilization is one of loss: loss of soil structure, soil carbon, ecosystem function, and diversity. As we find ourselves at the nexus of intersecting global challenges of radically altered biogeochemical cycles and anthropogenic climate and productivity influence, we urgently need to alter our relationship with the soil and biosphere that sustain our human systems. In this dissertation I evaluate two management strategies for enhancing soil organic matter (SOM) in dryland, grain fields in the U.S.: legume integration and perennial grains. These strategies have been part of traditional farming practices, but they are not commonly utilized by commodity farmers for reasons I explore in Chapter 5. I conclude with policy recommendations for one way that might lead to systemic change that would value soils and their vital role in our human systems more appropriately. Here I provide a brief synopsis of each chapter:

In the introduction (Ch. 1) I provide some historical context of human reliance on grain agriculture and the reasons that legumes and perennials might enhance SOM. I also describe the framework of SOM formation used in this research and provide an overview of the components of SOM I measured in this research.

The first study (Ch. 3) is an observational study of conventional, dryland wheat farmers in semi-arid Colorado and Nebraska. I examine the ‘soil carbon (C) dilemma’ (Janzen 2006):

How can SOM be increased, while also increasing the release of nutrients that accompanies decomposition? We specifically tested whether incorporating legumes into a continuous rotation influences the form and amount of SOM as well as productivity in farms of the central Great Plains region of the U.S. by contrasting three, no-till rotation systems: 1) conventional wheat-fallow; 2) continuous grain-only rotations, and 3) continuous grain rotations that incorporate a legume crop. We sampled on-farm fields and experimental agricultural research station plots that had received one of these rotations for at least eight years. We found that intensifying the rotation with continuous grains led to 1.5-fold increase in aggregate size but did not change SOC stocks. Incorporating a legume to the continuous grain rotation resulted in 1 Mg C ha^{-1} more SOC on average in surface soil compared to wheat-fallow rotations.

In chapter 3, I use a similar approach to assess whether conversion from annual to perennial grains such as intermediate wheatgrass Kernza® could sequester soil organic carbon (SOC). We sampled three sites with paired fields under annual grains and converted to Kernza 5-17 years ago to 100-cm and compared their SOC stocks as distributed between mineral-associated (MAOM) and particulate organic matter (POM). POM-C was higher under Kernza cultivation but total and MAOM-C were similar. Our findings suggest Kernza increases SOC at depth as POM. Further study is needed to assess whether this will result in long-term SOC sequestration.

In order to quantify the effect of legume incorporation and ability of Kernza to form SOC, I performed a mechanistic study to quantify the formation of SOM from Kernza and alfalfa tissues under contrasting N management (Ch. 4) Using continuously labeled $^{13}C/^{15}N$ plant residues, we tested the effect of litter inputs of contrasting composition (shoot and root material from Kernza® and alfalfa, a perennial legume) under management of Kernza where N was (1)

not added, (2) added as urea, or (3) fixed by an alfalfa intercrop. We selected Kernza for its theoretical potential to build SOM due to deep root systems and long growing season. We hypothesized that the higher quality litter from alfalfa shoots would lead to greater MAOM formation due to its higher density of metabolic components promoting enhanced microbial C use efficiency, while root tissues may more likely become stabilized within aggregates as oPOM due to increased contact with soil surfaces. We predicted that the management with N addition may enhance MAOM-formation by alleviating microbial N-limitation and leading to enhanced microbial C use efficiency. We found that overall Kernza promoted greater SOM formation, in both MAOM and oPOM, with 20% of roots stabilized and 12% of shoot stabilized after 27 mo compared to 10% for alfalfa roots and shoots.

Finally, in chapter 5, I propose a pilot crop insurance and research program in the U.S. Northern Plains to promote practices that enhance soil health, farm income, resilience, and mitigate climate change. Such a program could inform nationwide adoption of such practices.

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No human does anything alone, and though my fingers have been the ones stringing these words together, they have only been able to do so for the generosity, wisdom, patience, and support of many others. My advisor Francesca has been a consistent motivator and role model for the duration of this research effort. Many of the studies I carried out were inspired by her ideas, and most times we met I came away with a new idea, correction, or direction to take on a project. I've been inspired by how much Francesca invests in the success not only of her ideas but also in her students and colleagues. This work also would not have been possible without the support, encouragement, and feedback from my committee. When I look back at my ideas, writing, and projects from the start of this degree, I realize how generous they were with their praise and encouragement. I had so much to learn (and still do), and so appreciate how generous each of them has been with their time, energy, and feedback. It's amazing to me how when someone I admire expresses confidence in my abilities, I start to feel more confident. That's what Tim and Meagan have done for me consistently. Every time we met, I came away feeling enthusiastic not only about the project but also about my ability to do it. Turns out that's also important!

There are also so many people who've helped with the actual work described in this dissertation. Their names appear only in the acknowledgements sections of papers, but truly they helped ferry the knowledge from these data into the world. For their fieldwork brawn and perseverance, I'd like to give special thanks to Rebecca Even, Dylan Casey, James Bowdin, Brandon Peterson, the many interns and staff at The Land Institute and for the long-term research sites at Akron, Sterling, and the High Plains Ag Lab whom I never even met who tended the experimental plots I used for Chapters 2 and 4, and last but not least my nephew Henry Krieger. For anyone who can remember this far back… when the pandemic first started in March of 2020, field and lab work ceased for a while. The Colorado State University policy was that no humans were allowed to travel together in a vehicle, and there was an entire process for getting any field work approved. It would have been very difficult for me to collect the litter incubation (Ch. 4) samples by myself, but it also was not practical to rent multiple trucks for an 8-hour drive to Kansas. It also would have been impossible to follow CSU protocols for maintaining a 6 foot distance in the field since two people needed to work together closely to collect the soil samples. Henry to the rescue! Since I was not able to bring along anyone employed by CSU to help with the sample collection, my nephew got special permission from his school (entirely online at the time) to join me in the field for a few days in May 2020. He was an excellent technician. Probably one of the best I've worked with for his consistency, attention to detail, and stamina. Henry also helped process many of the samples in the lab where his skills proved just as valuable. I could not have completed that sampling without him.

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While I mentioned Rebecca before, I also feel compelled to honor her significant contribution to this dissertation. Not only did Rebecca help with so much of the sample collection for both Chapters 2 and 4, but she also performed the water stable aggregate analysis (Ch. 2) and microbial biomass analysis (Ch. 4). The final push to get this dissertation out the door meant that I did not include the microbial biomass data in this dissertation (stay tuned for a research publication), but she has added so much to the quality of the work I write about here. Not often do I find someone who is good at so many things – and that's Rebecca. Artistic, a singer, strong, persevering, fun to talk to, curious, and brilliant – I admire Rebecca and feel honored to be able to work with her for multiple projects. She taught me a lot about how to do things in the lab as well, for which I am forever grateful.

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The members of the Cotrufo and Agroecology labs have been an invaluable support and inspiration to me. How often do you get to work with so many brilliant and kind people? I appreciate the supportive environment and team mindset that each person brings in making our research better, helping each other improve, and supporting us individually. There was one time when I failed at a presentation (3 minute thesis contest) pretty fantastically though I had invested so much in preparing. Nora Flynn sent me a kind note afterwards. I do not remember exactly what she said at this point, but I remember she made a point to reach out and offer encouragement. That's the kind of people I am talking about – beyond being talented, being caring, genuine, and thoughtful. It's been a special honor to be part of these lab groups.

Then there are the people who've supported me on this journey. It seems odd to say, but this PhD is the longest I've held a single job. I came into this program naïve, and I am leaving it a better human. That's been made possible largely because of therapy which started with the CSU Health Network. I had a lot of healing to do unrelated to research or the graduate school. What the graduate school provided, however, was a place with the space and support to make real changes to myself. I had not had access to such a strong support system working with the US Coast Guard or teaching high school science, though I certainly needed help during those phases of my life, too. I would say the pressures of graduate school were no greater than any I had faced before, but they exposed me in ways I could not hide so easily and forced me to confront. I've had the privilege of working with a wonderful therapist who has helped me enjoy life in a way I never believed I deserved or thought possible for myself. I am certain without this support I would not have completed this dissertation. If I could have one wish for the world, it was that every person had this kind of opportunity and support if they wanted it. It has been the single best gift I take away from this graduate degree and will always carry with me.

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CHAPTER 1: INTRODUCTION

1. Beginnings: Grain-Powered Species

We are fundamentally grain-powered creatures. Humans began consuming calorie-dense grains (e.g., wheat, barley, corn, rice, etc.) and tubers (e.g., potato) as early as 100,000 years ago, long before any plants were domesticated. Evidence of intentional cultivation goes back 23,000 years to modern-day Israel where archeologists found people experimented with wild barley, oats, and wheat. What we would consider agriculture, though, began in earnest in multiple locations across the globe between 5,000 to 10,000 years ago (Smil, 2018). The cultivation and domestication of plants, animals, and ourselves (Zanella et al., 2019) supported higher population densities than our hunter-gatherer groups and necessitated greater expertise and recordkeeping than ever before. Ultimately, agriculture (e.g., grain cultivation) led humans to invent accounting, written language (Schmandt-Besserat, 2014), and increasingly complex systems of organization (Smil, 2018), setting the stage for the world we live in today.

I start us here, considering our roots as grain-powered beings, for context. Grains are so foundational to civilization, we easily take them and the soils they (we) depend upon for granted. Yet the history of civilization is brief compared to the history of soil. Soils form at a rate of 0.058 – 0.083 mm yr^{-1} (Montgomery, 2007); for reference, human hairs range from 0.05-0.12 mm in diameter. Over the past 23,000 years, roughly 1.3 to 1.9 m of soil formed while agriculture likely caused 7 to 10 m[1] of soil to erode over the past 12,000 years (Montgomery, 2007) releasing an estimated 116 Pg carbon (C) into the atmosphere (Sanderman et al., 2017).

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[1] Amount of erosion estimated based on the Montgomery (2007) calculation that erosion rates on cultivated lands are roughly 10 times the rate of soil formation. Estimate is for 12,000 years as a time when agriculture had its early beginnings (Sanderman et al., 2017).

The dissonance between soil formation and erosion in the most fertile areas on earth points to the nagging unsustainability at our core (Montgomery, 2012).

Our challenges do not end with the soil, however. We face intersecting and complex threats to civilization as a result of increasing consumption and degradation of natural ecosystems that have led to crises of biodiversity and ecosystem function loss, unprecedented alteration of biogeochemical nutrient cycles (Gruber and Galloway, 2008), and a rapidly changing climate (Steffen et al., 2015; Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), 2019). Being a grain-powered species, many of these challenges are intimately tied to agriculture (Foley et al., 2011).

We, a single species, directly consume between 20-28% of global productivity (Smil, 2015; Jenkins et al., 2020). Agriculture controls 49% of Earth's ice-free land area (12% as crops, 37% as pasture) (Ritchie and Roser, 2013). Prior to the dawn of agriculture >8,000 years ago, global changes to C stocks and primary productivity were driven primarily by changes to the climate due to fluctuations of incoming solar radiation and greenhouse gas concentrations (Smil, 2015). The replacement of natural ecosystems with less productive agricultural ones, however, has become a dominant force limiting global productivity. Conversion of forests and grasslands to croplands has reduced global C stocks stored in vegetation from its peak of ~1,000 Pg C roughly 8,000 years ago (c. mid-Holocene) to <650 Pg C today (Smil, 2015). Carbon lost from soils due to cultivation and grazing has increased atmospheric CO2 by 19% of pre-industrial levels of 600 ppm (Sanderman et al., 2017). Humans have also become a dominant force driving the climate with agriculture directly responsible for 12% of greenhouse gas emissions (IPCC, 2019) [33% if attributing land-use-change to agriculture (Wollenberg et al., 2013)]. While a daunting challenge, if we can figure out how to grow our food sustainably, we can go a long way towards aligning our collective lifestyle with what our planet can support.

What you will find within the following chapters is an exploration of two old ideas we could adopt more effectively than in the past to grow grains and shift our food system towards one that is sustainable. These ideas are "old" in that people have incorporated them into agricultural systems in some cases for thousands of years to sustain productivity and resources bounded by natural cycles, but largely colonial mindsets and technology have resulted in these practices falling out of favor (Carlisle, 2022). These practices, about which I elaborate below, are to incorporate legumes and perennials into cropping systems. I focus on how these might be adopted in the U.S. Central Great Plains, though the practices and policies could be applied broadly. I would not characterize either of these practices as a "solution" to the wicked challenges introduced by civilization; solving may be the wrong word when addressing wicked challenges (Skaburskis, 2008)—for as long as civilization functions, we will face these challenges and likely new ones we cannot yet conceive. I nevertheless argue that both of these ideas could help if we are able to incorporate them into our food system.

You might reasonably wonder what this dissertation contributes to the world if the ideas I explore here are "old". I argue that the practices are old, but our understanding of their influence on soil organic matter (SOM) and to modern (that is, industrial) agriculture have not been well-studied or appreciated. For example, legumes have been used as a source of nitrogen to soil as well as a nutrient-dense crop, but they have yet to be grown to enhance SOM. Furthermore, while perennial plants are the origins of all current crop species, perennial grains have not been technologically feasibly until recent decades. This is the first work to attempt to quantify the SOM formation of the emerging perennial grain crop Kernza®. Finally, I introduce novel approaches to study the effects these management practices have on SOM. For example, in Chapter 2 (van der Pol et al., 2022b) I combine farm-based measurements with a mechanistic model to extend our understanding of the soil organic carbon (SOC) dynamics. Part of the value in these types of studies is that, while we know these practices likely benefit the soil, we now know more precisely *by how much*. In a world of funding and policy and the urgent need to protect our soils and reduce greenhouse gas emissions, this *by how much* will aid decision-making about how to allocate resources and priorities for where and how to invest our limited resources. I

This is an outline of the studies that follow: Chapter two focuses on the U.S. Central Plains and explores the potential for intensification and legume integration to 'solve' the soil C dilemma by rebuilding SOM while maintaining productivity through an observational study and model. Chapter three compares the ability of the perennial intermediate wheatgrass being domesticated for grain production as Kernza® to build SOM compared to annual grains. Chapter four is a mechanistic study of SOM formation from root and shoot tissues of Kernza and perennial legume-alfalfa (*Medicago sativa*) under contrasting nitrogen (N) management to assess the ability of Kernza to accrue SOM when grown as a monoculture and unfertilized biculture. Finally, Chapter five synthesizes policy recommendations for one way that we could shift part of the USDA crop insurance program and funding to remove some of the institutional barriers to farmers adopting new practices that could transform our agricultural system.

2. Background: Historical context and rationale for studying soil organic matter

The management practices studied in this work are not novel in themselves, but the current context of agriculture has changed. The cultivation of legumes to maintain soil fertility appears early in the agricultural record (c. 7,000 years ago) (Stinner et al., 1992). And while none of the grains that account for 80% of our food supply are perennial (Pimentel et al., 1986, 2012), the ancestors of these crops were (Van Tassel et al., 2010). The reason to study and discuss these practices now is both the urgent need to reduce the environmental impact agriculture has on Earth's vital systems (e.g. climate, nutrient cycling, biodiversity) and the technological capacity to make perennial grains a viable possibility.. In a world with synthetic fertilizers and herbicides, specialized machinery that allows small numbers of people to manage vast acres of land, and technologies that greatly accelerate crop breeding and domestication (Cox et al., 2002, 2004), there are more options. In the case of legumes, technological alternative sources of N have reduced legume cultivation (Crews and Peoples, 2004), but in the case of perennial grains, technological advances makes their existence possible (Van Tassel et al., 2010).

How we got here: the invention that led to dis-integration

Successful early agricultural societies were circular: nutrients were recycled among harvested crops, humans and livestock, and the soil (Crews, 2005). Without fertilizers, power tools, or a mechanistic understanding of how plants grow, people maintained the soil fertility by practicing a variety of ways to return nutrients harvested to the soil and protect soil from erosion. Chief among these strategies were the cultivation of legumes such as lentils, alfalfa, clover, and peas, allowing animals to graze fields after harvest, and adding nutrient-rich materials such as compost, seaweed, and even human waste to fields (Smil, 2018). Legumes promote soil health largely due to their special ability to access atmospheric N through biological N-fixation (BNF) (Vitousek et al., 2013). The most successful of these systems were highly productive, diverse polycultures which accrued organic matter over time, recycled nutrients, and minimized erosion (Crews, 2005). Until as recently as the 1950s, farmers commonly sustained 25-50% of their farm under a legume pasture or cover crop to regenerate soil fertility (Crews and Peoples, 2004) despite the relatively low biomass of most leguminous plants (Monfreda et al., 2008; Iizumi et al., 2014).

Agriculture's departure from the integrated, cyclical model it followed for millennia began in the 1840s when early European agronomists such as Justus von Liebig determined that adding N to crops could increase production (Smil, 2004). Rather than conceive of ways to enhance N recycling through cultivation, Liebig and others promoted a reductionist view that relied on importing materials from outside the system (Manlay et al., 2007) – even from thousands of miles away. Early fertilizers were naturally occurring and rapidly depleted deposits of bird guano and nitrogen-rich rocks (caliche) off the coast of South America (Smil, 2004). Caliche was also used by Allied forces during World War I to make explosives, and it was Germany's realization that it could and would be cut off from this resource so vital to weapon-making that prompted the country to invest in research to develop what is arguably the most consequential invention humanity and the Earth has yet known: the Haber-Bosch process (Smil, 2004; Erisman et al., 2008).

The Haber-Bosch process is an industrial method for fixing atmospheric N (hence, INF), into the biologically usable form of ammonia. Since its invention, the Haber-Bosch process revolutionized both ammunition production and agriculture. As such, INF can be directly attributed to the deaths of hundreds of millions of people in armed conflict (Erisman et al., 2008) while also making possible half of people alive today and an estimated 5.5 billion more people by 2050 (Smil, 2004; Crews and Peoples, 2004).

Since the 1950s when commercial fertilizers first became widely available, fertilizer application has increased 8-fold while the proportion of BNF-derived N in food has fallen from

50% to 20% (Crews and Peoples, 2004). This shift has greatly altered the energy inputs in agriculture. N-Fixation, both biological and industrial, is energy-intensive due to the intrinsic molecular properties of N_2 gas. While BNF is solar powered, however, INF is powered by fossil fuel combustion. The energy demands are such that fertilizer synthesis alone accounts for 1% of global energy use (Smil, 2004).

Commercial availability of synthetic fertilizers rapidly accelerated the shift from circular, solar-based agriculture to linear, fossil fuel based systems, but these practices were institutionalized in the US in part through the Federal Crop Insurance Program (Hamilton, 2020). The unstable markets of the Great Depression (1933) and the unsustainable practices of frequent (7-8 times per year) tillage coupled with a decade-long drought that became the Dust Bowl (1936) led to many farmers to need disaster assistance and many more to lose their land (Peterson et al., 2020). As part of the New Deal under President Franklin Roosevelt, crop insurance was created as a means to support rural economies, ensure national food security, and stabilize food prices.

The program was unpopular at first, and hemorrhaged taxpayer funding until the 1950s when the federal government paid bankers a commission when they issued a loan to a farmer with insured crops (Hamilton, 2020). In this way crop insurance tied new technologies that enhanced production (fertilizer, herbicide, more sophisticated (fuel-powered) equipment) to the financing needed to acquire these production-enhancing tools and institutionalized what Willard Cochrane termed the “technology treadmill” (Levins and Cochrane, 1996).

The treadmill begins with farmers adopting technology that increases their production, giving the early-adopters a profitable edge. As more farmers adopt the new technology, however, food supply increases and prices fall, diminishing the profits of everyone involved. To stay viable, farmers are forced to acquire more land and newer technology to ‘stay ahead’, requiring more financing (loans) to acquire new technology to sustain the high productivity levels needed to repay the substantial loans used to increase productivity in the first place (Hamilton, 2020).

This drive towards increasing productivity and higher yields has contributed to food systems that are less diverse as 90% of food comes from just 15 crop species (Pimentel et al., 1986). Grain production today is deeply dependent on fossil-fuel energy both for fertilizers as well as all aspects of management, and farms tend to be less resilient (Matson et al., 1997) both to extreme weather as well as to the economy (Annan and Schlenker, 2015; Altieri et al., 2015; Wienhold et al., 2018). In the U.S., grain farmers increasingly rely on subsidy programs such as crop insurance to survive market and weather volatility (Schipanski et al., 2016; Tack et al., 2018) while few support mechanisms exist outside a handful of commodity crops. In the Great Central Plains of the U.S. where the studies in this work are based, this cycle has resulted in a cycle of decreasing profitability as net farm income has fallen by half since 2013 (USDA-ERS, 2017). Full-time farming has become rare while farm size has grown (Carolan, 2016) and per area consumption of reliance on synthetic fertilizers for nutrients and herbicides to manage weeds has increased (Anderson, 2003).

The availability of INF-N along with the indefatigable work of crop breeders such as Norman Borlaug (Mann, 2018) have transformed agriculture and altered the trajectory of the human population (Smil, 2004). Since 1961, the total number of calories produced has increased faster than population growth (global average food production per person 1961: 2189 kcal person^{-1} day^{-1}, 2017: 2884 kcal person^{-1} day^{-1}). Grain productivity per hectare has increased 2.2-fold, and the proportion of people who are undernourished halved since 1990 (one billion people or 19% to 800 million people or 11% of global population) (Gordon et al., 2017). In doing so, however, we have decoupled biogeochemical cycles from their natural processes to highly uncertain effect (Gruber and Galloway, 2008). We are now at a juncture where the toll of humanities' increasing appropriation of natural resources is evident (Smil, 2015; Steffen et al., 2015). A transformative shift in how we grow our grains is needed to reverse soil degradation (Jang et al., 2021) and loss of SOM (Guo and Gifford, 2002; Sanderman et al., 2017), while mitigating the numerous harmful effects of eutrophication, air and water pollution, species mass extinction, acceleration of anthropogenic climate change (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), 2019), increasing inequality, and persistent malnutrition (Gordon et al., 2017). Legumes and perennials are two paths towards that goal. While there are benefits and consequences of pursuing each of these pathways that extend beyond the farm (*for legumes see*: (Jensen and Hauggaard-Nielsen, 2003; Plaza-Bonilla et al., 2017; Iannetta et al., 2021); *for perennials see:* (Streit Krug and Tesdell, 2021; DeHaan and Van Tassel, 2022a), in this work I have focused on how incorporating legumes and perennial grains into grain agroecosystems influence soil organic matter.

The importance of soil organic matter (SOM)

Soil organic matter (SOM), roughly half of which is made of carbon (C), is a relatively small portion of mineral soils (>1-10%), yet it is critical to ecosystem services provided by soils including improving soil structure for plant growth, holding and filtering water, and retention and provision of nutrients (Smith et al., 2015; Baveye et al., 2016; Yan et al., 2020). Because it is central to soils' function, especially in an agricultural setting (Oldfield et al., 2019; Kane et al., 2021), is responsive to management (Janzen et al., 1997; Cotrufo and Lavallee, 2022), and tends to be lost as a result of human activities (Guo and Gifford, 2002), we tend to focus on ways to get it back or assess how much has been lost. And though C constitutes a relatively small portion of most soils (the exception being organic soils which are not present in the study areas in this work), the pool of C globally is substantial: soils store three times more C than the atmosphere and vegetation combined on the order of 2344 Pg C in the top 3 m of soil globally (Jobbágy and Jackson, 2000; Schmidt et al., 2011). Given the losses of SOC due to human activities (Sanderman et al., 2017) and the urgent need to reduce atmospheric C to mitigate climate change (Lee et al., 2021), accruing SOC in managed lands could have a co-benefit of reducing atmospheric CO_2 (Bossio et al., 2020; Amelung et al., 2020) and promote cooling (Girardin et al., 2021).

A question naturally follows the importance of SOM and the quest to regenerate it: how does SOM form and persist? Our understanding of SOM dynamics continues to evolve. Early conceptualization of SOM were that molecules underwent microbial processing and became increasingly complex both chemically and physically until they could no longer be decomposed by the microbial community (e.g., ‘humification’) (Balser, 2005). (*For a full critique of this model, see* (Lehmann and Kleber, 2015). With improved methods for quantifying SOC and N, however, this view has been replaced with understanding that SOM formation is the result of complex interactions between soil minerology, particle size, climate, plant and microbial communities, and climate. Soil scientists and ecologists describe SOM persistence as an “ecosystem property” with the understanding that the reason SOM accumulates or decomposes is the result of the system it is in rather than any single factor or molecular structure (Schmidt et al., 2011). The complexity of SOM dynamics makes it difficult to predict how, when, and under what conditions SOM will accumulate and persist, hence the profound research investment in improving our understanding.

Views of how and why SOM forms and persists

In the past 15 years, many conceptual frameworks have emerged to conceive of SOM formation (Grandy and Neff, 2008; Cotrufo et al., 2013, 2015; Lehmann and Kleber, 2015; Sokol et al., 2019; Waring et al., 2020; Daly et al., 2021). These frameworks vary in their emphasis on the timing, location, and movement of C through various pools of SOM, though they share an attempt to integrate the dynamics between mineral surfaces, plant tissues, soluble compounds, and the microbial community. Most of these frameworks characterize SOM broadly into three pools of OM: particulate (POM), mineral-associated (MAOM), and a third ephemeral (process-based) category of soluble (alternately *dissolved* or *water-extractable*) OM (Lavallee et al., 2020). This characterization of SOM has proven useful for understanding SOM dynamics (Cotrufo et al., 2019; Haddix et al., 2020) as it partitions SOM into measurable pools with distinct chemical and physical properties in a way they can be linked to potential pathways of formation and persistence (Cotrufo and Lavallee, 2022).

The studies in this work follow the two-pathway model of SOM formation (Cotrufo et al., 2015). This model posits that when plant material first begins to decompose, the concentration of soluble (metabolic) components are high and readily leached into the soil as dissolved OM (DOM); a similar process could take place for root exudates (Sasse et al., 2018). Being of small molecular size and metabolizable, these compounds may sorb directly to mineral surfaces or be consumed by soil microbes which, in turn, may exude enzymes or waste products that form bonds with soil minerals(Kleber et al., 2015). In this framework, MAOM tends to form early-on in the decomposition process and to a lesser extent as decomposition progresses and the quantity of exchangeable chemical bonds and quantity of soluble material available for sorption onto minerals declines over time. While the soluble components of plant tissues may be mobilized quickly and readily, the structural components (celluloses, lignin, cutin, suberin) are physically larger and insoluble and thus are less mobile in the soil. These components lead to the physical SOM formation pathway that leads to POM. As soil fauna communize plant tissues, litter fragments and is gradually incorporated into soil as POM. That POM may remain "free" (fPOM) – that is not bound within soil structures – or it could be occluded (oPOM) within aggregates where it may be protected from decomposition due to the physical and chemical conditions within aggregates slowing decomposition (Schrumpf et al., 2013; Upton et al., 2019). POM can also be measured based on its density where the "light" POM (lPOM; typically density <1.85 g cm^{-3}) tends to be the relatively undecomposed plant material, and "heavy" POM (hPOM or sometimes 'heavy-course' OM hcOM; typically density >1.85 g cm^{-3} and size > 53 µm) may be adhered to sand-particles and likely has undergone some microbial processing (Leuthold et al., 2022).

Under this framework, litter chemistry is thought to play a significant role in the fate of plant-C into SOM. Plant tissues with a higher proportion of metabolic components (labile compounds), that exude a greater quantity of their photosynthate through roots, and that have higher N-concentration would likely contribute to the MAOM pool to a greater extent due to the plant metabolites forming MAOM directly and indirectly though efficient microbial processing (Cotrufo et al., 2013). Since soil microbes have a higher demand of N than plants [microbe C:N ~8, plant tissues ~45-60 (Soong et al., 2020)], increasing the N-content of plant tissues can improve the C-use-efficiency of the microbial community since they would need to degrade fewer molecules to obtain sufficient N to meet their growth requirements (Kallenbach et al., 2019). This 'microbial filter' is one factor of SOM stabilization within the 'Microbial-Efficiency Matrix Stabilization (MEMS)' framework, with the other major component being the 'matrix' or the underlying soil texture and minerology. Soil minerals vary widely in their chemical structures and ability to form bonds with organic materials as has been demonstrated in the lab (Mikutta et al., 2006; Singh et al., 2016) and field studies (Mikutta et al., 2019), and thus the soil itself imposes a ‘mineral filter’ on SOM formation. A study integrating the microbial and mineral filter concepts examined SOM formation and protection on contrasting iron and aluminum phases across a range of soil orders and found DOM helped bind soil particles with metal oxides to form stabile organo-mineral complexes (i.e. MAOM) with lower C:N compounds forming stronger bonds (Wagai et al., 2020). In this work, the role of litter chemistry in SOM dynamics is considered in annual (Chapter 2, van der Pol et al.,2021) and perennial (Chapter 4) crops, and soils within each experiment were controlled to the extent possible for the influence of the underlying minerology (Chapters 2-4).

Following the contrasting origins of the MAOM and POM fractions, each fraction has distinct characteristics. POM, often measured simply as >53 µm, tends to have a higher C:N owing to its plant origins and a short residence time (<1 – 10 years). There is no theoretical limit to POM accumulation as it is unrelated to soil texture; its presence and turnover are dictated primarily by inputs and how favorable conditions are for decomposition (Cotrufo et al., 2019). MAOM constitutes the majority of SOC in mineral soils, especially in agricultural systems including those in this work where MAOM-C may be >90% of SOC (Sokol et al., 2022); the amount of MAOM is, in part, determined by the proportion of silt and clay minerals in the soil and could potentially “saturate” or fill up all available mineral bonding sites on the soil particles (Six et al., 2002; Castellano et al., 2015). MAOM, often measured as <53 µm, has a lower C:N ratio due to its microbial and soluble compound origins, and outside of microbial hotspots tends to persist, on average, four times longer than POM (Lavallee et al., 2020; Lawrence et al., 2020; Cotrufo and Lavallee, 2022).

Soil fractions are parsed and measured in a variety of ways (Poeplau et al., 2018; Leuthold et al., 2022) that typically involve a water-extraction, size-based separation, or a combined size and density separation. Fractions of SOM protected within aggregates can also be measured after aggregate dispersion (Fulton-Smith and Cotrufo, 2019; Haddix et al., 2020) or the aggregates dispersed and then POM/MOAOM separated (Mosier et al., 2021). The fractionation scheme chosen is a balance between the research question(s) of interest and the cost as having more fractions to quantify increases the cost of analysis. In this work, I used three different fractionation schemes across each research chapter. In Chapter 2 (van der Pol et al., 2021), the fractionation scheme was chosen to match the modeled pools in the MEMS v1.0 model (Robertson et al., 2019) where I performed a size and density fractionation into DOM, lPOM, hPOM, and MAOM. In Chapter 3 (van der Pol et al., 2022) and 4, I used the simplest size-based fractionation of MAOM and POM. Finally, in Chapter 4, I also conducted a size-density fractionation to separate fPOM, from oPOM, and MAOM in order to estimate the degree of protection of litter-derived C.

3. Pathways forward: Rethinking two old ideas in agriculture to increase SOM

Pathway 1: What is the potential for legumes to affect SOM formation and persistence?

Legumes could alter SOM formation and C-sequestration through a variety of pathways specific to common lifestyle and growth traits as well as their ability to increase ecosystem N. Legumes tend to have relatively high N content in their tissues giving them a lower than average C:N (typical may be 25:1 for legumes vs. 60-80 for corn and wheat or >400 for woody species). The high concentration of N is poorly correlated with photosynthetic capacity, however, and seems more related to the requirement for a high N concentration in leguminous seed pods (giving them high nutritive value) (Vitousek et al., 2002). There is some debate also about the relatively high demand for phosphorus (P) in legumes, whether that is also a life-style trait or a physiological constraint for facilitating biological N fixation (BNF) in their nodules (Vitousek et al., 2002). The low C:N ratio of legume tissues and the high proportion of N in root exudates (accounting for 13-16% of BNF-N (Carranca et al., 2015) lends the legume residue to be efficiently metabolized by soil microbes meaning that relatively little of the C is respired away through decomposition compared to lower-quality residues with a higher C:N (Cotrufo et al., 2013).

In addition to their high quality tissues, living legume roots may manipulate the microbial community to favor organisms that support plant-growth promoting and microbes with a tendency towards higher carbon-use-efficiency (CUE). Some have found that the *Rhizobia* bacteria many legumes cultivate in their nodules for BNF also may secrete plant-growth promoting factors, supporting the growth of neighboring or intercropped plants and aiding in disease suppression in the soil (Stagnari et al., 2017). Additionally, many legumes facilitate colonization of arbuscular mycorrhizal fungi (Galvez et al. 1995; Douds et al. 1997; N.Z. Lupwayi et al. 2007), enhancing the phosphorus mobilization in the soil. The increased presence of fungi may support higher CUE of decomposing litters as well as increased soil aggregation that is often associated with legumes (Kumar et al., 2018, ch. 4). Aggregation may provide a physical pathway to preserve both POM and MAOM by making the OM inaccessible to further decomposition in limiting water or oxygen environment.

Legume cultivation studies, particularly those with intercropping, tend to show increased SOM from the legume cultivation (Binkley and Menyailo, 2006; Duchene et al., 2017;

Drinkwater et al., 2021). A study comparing SOC stocks after pea and lupine cultivation as well as with maize monocropped and inter-cropped with soy found that in instances with a legume, SOM was enhanced (Bichel et al., 2016). Additionally, applying soybean residue increased SOC 38% (Bichel et al., 2016). Similarly, a study of how plant composition influences SOC found that when grasses were coupled with a legume they enhanced SOC by 522% relative to a monoculture; the SOC increase was attributed to enhanced legume root biomass and rooting depth (Fornara and Tilman, 2008). Several studies have shown that most soil C may be derived from root C (Rasse et al., 2005; Fry et al., 2018) and that deeper roots tend to lead to greater SOC (Lange et al., 2015; Poirier et al., 2018).

Other studies have compared legume residue to that of other crop species, often with mixed effect whether the legume was grown in place or added as a mulch. For example, one study found that neither adding a legume-corn mulch nor incorporating legumes into a rotation with corn had an effect on SOC, but that corn mulch alone increased SOC (Chen et al., 2018). Another study which combined lab and fields experiments contrasted the effect of litter quality (including a legume) on SOC and found no differences (Gentile et al., 2011). Others studies with legumes as part of a rotation have found legume tissues to be preferentially retained in soil (Drinkwater et al., 1998) as well as higher initial retention but no overall enhancement to SOC after two years (Carranca et al., 2009b).

In all, it seems we know that legumes grown as an intercrop tend to promote SOM accumulation, likely as a result of increasing root biomass and rooting depth. Some studies have found, that legumes sometimes have a negative effect on root density (Lange et al., 2015), however, which may contribute to their reduced soil C inputs compared to cereals in some instances (King and Blesh, 2018). Grown as a grain in rotation, legumes may enhance SOM, possibly by providing both labile substrates that can be more efficiently stabilized as MAOM as well as by promoting aggregation through enhanced fungal growth. These mechanisms, however, have not been thoroughly investigated. Finally, as described there are many instances when incorporating legumes has no long-term effect on SOC , though it is less clear why this may be.

Compared to commodity grain crops, legumes have higher nutrient density (Iannetta et al., 2021), lower environmental footprint (Plaza-Bonilla et al., 2017), and multiple benefits to the soil structure and nutrient cycling (Stagnari et al., 2017). As such, integrating legumes into any crop rotation provides a suite of potential benefits regardless of their effect on SOC (Duchene et al., 2017). The high proportion of labile components and N in legume tissues provide an opportunity to test the MEMS framework and importance of litter chemistry to SOM formation. The results have implications for regenerating SOM in grain systems which could enhance farm resilience and rebuild soil health. I explore the potential for legumes to enhance SOM in annual dryland grain farms in Colorado and Nebraska using an observational study and MEMS v1.0 model in Chapter 2 (van der Pol et al., 2021). I return to the potential for legumes to enhance SOM formation in Chapter 4 where I quantify SOM formation from legume alfalfa and study the influence of a legume intercrop vs. fertilized monoculture on SOC.

Pathway 2: What is the potential for perennial grains to influence SOM formation and persistence?

Perennialization is frequently promoted as a strategy to enhance SOC as well as increase food and ecosystem security (Glover et al., 2010b). The rationale behind the benefits of perennial crops compared to annual ones are based on the enhanced ecosystem services and function of perennial systems compared to annual (Glover et al., 2010a; Crews and Rumsey, 2017; DeHaan and Van Tassel, 2022b). Perennial crops epitomize several of the widely accepted best-management practices that tend to enhance SOC (Lehman et al., 2015; Paustian et al., 2019) including minimizing disturbance, increasing the presence and amount of time with living roots are in soil, and increasing root biomass and depth. Currently perennial crops, defined as crops that are planted but not replanted annually, cover 30% of global croplands (Ledo et al., 2018) and are typically categorized as woody plants (e.g. fruit/nuts - blueberry, apple, cashew; beverage – coffee, cacao; oil crops – palm) or grasses (e.g. food – sugarcane, rice, Kernza; bioenergy –switchgrass) (Ledo et al., 2020). Perennial grains such as Kernza and rice are so recent and their cropped area comparatively small that they are not yet included in global studies of SOC. While results of many studies about the effects of a perennial crop on SOC are varied (Lal, 2004; Qin et al., 2016; Crowther et al., 2016; Sprunger et al., 2018a), this is often attributed to the difference among crops and geographic areas (Ledo et al., 2020) rather than the potential for perennial crops to influence SOC. A study that evaluated change in SOC from annual to perennial crops at a global scale found that SOC generally increases by 20% 0-30 cm and 10% 0-100 cm over 20 years, and that SOC gains were greatest for woody crops (Ledo et al., 2020). The chief mechanism for the increased SOC is thought to be greater input to the soil from increased plant production and root growth as roots are consistently found to be preferentially stabilized in the soil (Rasse et al., 2005). Supporting this hypothesis is that the same global study found that conversion from pasture to a perennial crop resulted in a decline in SOC (1% 0-30 cm and 10% 0-100 cm) (Ledo et al., 2020). These global estimates were dominated by woody crops, however, which may accrue SOC fundamentally differently than grass crops since they are long-lived, slow to establish, produce substantial woody biomass (high C:N), and have a small portion of the plant harvested each year.

Perennial grains being grass crops might influence the SOC in ways analogous to grasslands and prairies, where the dominant form of SOC is in MAOM and SOC accrual is thought to be largely from root-C (DeHaan and Van Tassel, 2022b). Converting annual crops to perennials offers the greatest potential to restore much of the SOC lost from agricultural soils (Lal, 2004) by increasing SOC inputs relative to the annual system. Though SOC gains would not be indefinite (Johnston et al., 2009; Smith, 2014), they could be substantial. An estimate of the global SOC sequestration potential if annual grain, oil, and fiber crops were converted to perennial varieties found the global C sequestration could be 0.4-1.8 Pg C yr-1 (13-54 Pg C) within 30 years of conversion to perennial grain agriculture (Crews and Rumsey, 2017). These estimates are within range of other studies of soil C sequestration potential (Sommer and Bossio, 2014), though they are poorly constrained. The studies in this work focus exclusively on the perennial wheatgrass *Thynopyrum intermedium* grown for the grain Kernza which is currently the only perennial grain available to farmers in the U.S., though there are many perennial grains under development worldwide (Soto-Gómez and Pérez-Rodríguez, 2022). Studies of SOC accrual under Kernza fields have had mixed results (de Oliveira et al., 2018; Sprunger et al., 2018a). Kernza grown as a monoculture is currently the dominant practice on farms, though there's potential for Kernza intercropped with a legume to rely substantially less on fossil energy inputs and have greater resilience and ecosystem function (Crews et al., 2022). Could a Kernza-intercrop accrue more SOC than one supported by synthetic N? I explore the ability of Kernza to accrue SOM relative to annual crops in Chapter 3 (van der Pol et al., 2022) and seek to quantify the ability of Kernza to contribute SOM both as a monoculture and legume-intercrop in Ch. 4.

4. Growing deeper: policy implications

While there are unknowns about the potential for legume integration and Kernza to support increased SOM, there are many known benefits to cultivating legumes and perennials. The question remains, why are not more farms cultivating legumes and experimenting with perennials? Legumes tend to be less profitable than commodity grains, as they tend to have lower yields and fetch a lower price (Reckling et al., 2016). When operating on a low profit-margin or reliant upon a subsidy program such as crop insurance, some farmers may not be able to afford to grow a crop that is not one of the few eligible for insurance or which fetches a reduced premium. The risk would be even greater to grow a crop like Kernza which currently has yields one sixth that of wheat (Jungers et al., 2018) and which requires significant effort to get to market (Soto-Gómez and Pérez-Rodríguez, 2022). The choices farmers make are influenced by more than economics or environmental benefits (Carlisle, 2016; Rosenzweig et al., 2020), however, though these are both important. Given the urgency of the crises we face as a species, the best question is not why farmers do what they do, but rather what is the system that we need in this moment and how do we get there?

In Chapter 5 (van der Pol et al., 2021) I advocate for a policy proposal for the U.S. that could feasibly be implemented based off of current USDA funding and program structure of the Federal Crop Insurance Program. I argue for three changes which I believe could enhance the crop diversity of U.S. farms which, in turn, would likely also result in a cascade of improvements from soil health through increased SOM to economic and environmental resiliency.

Incorporating legumes as intercrops, forage, or cover crops requires recognizing the mutualistic relationships among legumes, soil, and other plants and participating in management practices that consider these interactions holistically rather than as a transactional economic exchange. Creating a world less reliant on INF-N may require creating a society that values these mutualistic connections and considers decision-making within a global context of possible downstream effects.

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CHAPTER 2: ADDRESSING THE SOIL CARBON DILEMMA: LEGUMES IN INTENSIFIED ROTATIONS REGENERATE SOIL CARBON WHILE MAINTAINING YIELDS IN SEMI-ARID DRYLAND WHEAT FARMS[2]

1. Introduction

Enhancing soil organic matter (SOM) in semi-arid agroecosystems as they face increasing anthropogenic pressures is a crucial adaptation strategy to sustain food production in these regions (Plaza-Bonilla et al., 2015). Additionally, SOM accrual results in increased soil organic carbon (SOC) storage, which is considered a powerful management strategy to combat climate change (Bossio et al., 2020).

Semi-arid regions, defined as areas where the aridity index (ratio of mean annual precipitation (MAP) to mean annual potential evapotranspiration) is between 0.2 – 0.5, cover roughly 20% of Earth's terrestrial surface (Koohafkan and Stewart, 2012). These regions supply about one half of global wheat production (O'Leary et al., 2018), are home to more than 1 billion people worldwide (Reynolds et al., 2007), and face many threats exacerbated by climate change. Threats include soil degradation (Hoyle et al., 2013; Koohafkan and Stewart, 2012), reduced yields from rising temperatures (Zhao et al., 2017), and increasingly scarce and variable precipitation (Ahlström et al., 2015) potentially leading to soil C loss (Delgado-Baquerizo et al., 2013).

Productivity in semi-arid regions is ultimately limited by MAP (Ahlström et al., 2015), presenting a challenge to management efforts to increase SOM. The common practice of summer fallow (a 14-month fallow between winter wheat plantings) is used to store soil moisture to

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stabilize wheat yields. However, removing fallow periods can improve overall system water-use-efficiency and have a positive effect on annualized crop yield (Peterson et al., 1996; Sherrod et al., 2014) and SOC (Hoyle et al., 2013; Peterson et al., 2020). This explains the U.S. Northern Plains regional trend that grain farmers have pursued in recent decades to eliminate the 'summer fallow' in favor of continuous cropping where a crop is grown annually (Rosenzweig and Schipanski, 2019). Continuous cropping in continental climates improves the water-use-efficiency since crop growth coincides with the majority of precipitation in summer (Peterson et al., 2020), while fallow results in minimal (<40%) soil water storage (Peterson et al., 1996). The trend towards annual cropping not only improves crop yield and farm income (Rosenzweig and Schipanski, 2019), but it may also lead to SOC gains and improvements in soil structure through increased aggregation (Rosenzweig et al., 2018; VandenBygaart et al., 2010).

While intensification has been well-documented as a strategy to increase SOM, less attention has focused on whether the crop type impacts SOC accrual and other desired SOM functions. In this study we expand upon previous studies of intensification to ask: How do the types of crop used to intensify from wheat-fallow to continuous rotations influence the amount and form of SOM accrual and overall productivity with respect to yield and fertilizer efficiency? This question is central to the long-term viability of agriculture in the region under rapid climate change and the potential for reduced reliance on energy-intensive fertilizers (Plaza-Bonilla et al., 2017). Increasing crop inputs typically leads to increased SOC (Chappell et al., 2016; de Deyn et al., 2008). However, this is not always the case because fresh inputs may prime the decomposition of native SOM (Poffenbarger et al., 2020). In contrast, there are also cases where there have been greater than expected SOC increases based on C input in systems that incorporated legumes into the management practice attributed to greater SOM formation from legume residues (Drinkwater et al., 1998; Gregorich et al., 2001). These contrasting results suggest that different types of residue input may have different results on SOC stocks. Additionally, SOC can be found in contrasting SOM fractions, and the formation of different SOM fractions may lead to different outcomes (Lavallee et al., 2020).

Growers face a dilemma related to the form and nature of SOM. Janzen (2006) described the inherent trade-off of SOM-accrual in agricultural systems as the "soil C dilemma", whereby the benefits of SOM to a grower come in part from its decomposition as that releases essential nutrients such as N for plant growth, but once decomposed, the SOC is lost to the atmosphere as CO_2 contributing to climate change. Farmers have a compelling need both to 'hoard' and 'use' SOM – but does one condition preclude the other?

We propose that solving this dilemma requires considering the form of residue input to soil. Specifically, we suggest that the relative contribution residue input has to SOM fractions with contrasting characteristics, chiefly particulate (POM) and mineral-associate (MAOM) organic matter (Lavallee et al., 2020), determines the magnitude and direction of change to SOM. While both these forms of SOM are dynamic (Kleber 2015), the mechanisms and controls of their formation and persistence differ (Cotrufo & Lavallee, *in press*). POM is derived primarily from structural components of plant tissues, and unless contributed by pyrogenic organic matter or protected within fine aggregates, it turns-over relatively fast (<50 yr) and is vulnerable to disturbance in agricultural mineral soils (Lavallee et al., 2019). POM has a variable C:N (10-40) depending on vegetation type (Lugato et al., 2021). Thus, in systems with low C:N inputs POM turnover can sustain fertility and promote crop growth. In contrast, MAOM originates primarily from metabolic components of plant tissues as well as exudates adsorbing directly to mineral surfaces, or after being transformed by microbes (Lavallee et al., 2020;

Cotrufo et al., 2015; Liang et al., 2017). While a fraction of MAOM exchanges readily, most of it is highly protected by the mineral association, resulting in lower vulnerability to change and longer mean residence time in soil than POM (Rocci et al., 2021; Cotrufo & Lavallee, *in press*). We propose that the contrasting origins and behavior of POM and MAOM is a powerful determinant of the potential for management to enhance SOM and farm productivity in grain systems. We hypothesized that intensifying and diversifying a rotation influences the form and amount of SOM accrual, and that managing to sustain POM turnover while increasing inputs and MAOM stocks solves the soil C dilemma. In accordance with the Microbial Efficiency Matrix Stabilization (MEMS) framework, one way to increase MAOM-formation would be to introduce relatively labile (low C:N & highly soluble) inputs that could adsorb to soil minerals directly after being efficiently metabolized by soil microbes (Cotrufo et al., 2013). This framework has been demonstrated experimentally (e.g. Cotrufo et al., 2015; Haddix et al., 2016) as well as expressed as a mechanistic model with measurable C pools (Robertson et al., 2019; Zhang et al., 2021), though not yet tested with an integrated approach which uses SOM measurements to validate model simulations, improving our ability to detect the direction and form of SOM changes over time. We use this integrated approach by combining measurement and model simulation to test our hypothesis in this study.

Legumes have a lower C:N ratio and a higher proportion of metabolic and low molecular weight compounds and thus they may result in both generation of fertility through the turnover of the POM, and efficient MAOM formation (Cotrufo et al., 2015; Haddix et al., 2016). This could sustain productivity while achieving longer-lasting SOC gains than intensification with grain crops alone, which we expect to contribute primarily to the formation and turnover of POM, but without much N recycling or MAOM accrual. Some farmers in the central Great

Plains of Colorado and Nebraska have created an opportunity to test this framework by incorporating peas (*Pisum sativum*) into their continuous rotations which also include wheat (*Triticum aestivum*), and some combination of corn (*Zea mays*), millet *(Panicum miliacium*), sunflower (*Helianthus annus*), and/or sorghum (*Sorghum bicolor*). Legumes have traditionally been cultivated to maintain soil fertility due to the recognition that they provide increased yields to subsequent crops (Angus et al., 2015), but their use in grain systems has declined since the 1950s with the advent of synthetic fertilizers (Crews and Peoples, 2004; Preissel et al., 2015). However, the relatively low biomass of legume crops compared to grains (Preissel et al., 2015) and the low frequency of being grown (once every three to four years), could diminish any measurable effect of peas in this crop rotation especially in semi-arid regions prone to crop failure due to drought and hail (Oldfield et al., 2019). The effect of intensification and incorporation of legumes on SOC and N stocks, distribution across SOM fractions, and crop productivity has not previously been studied at the field-scale to our knowledge. Understanding the potential for this management practice to enhance SOC sequestration and crop productivity will inform the extent to which minor changes to cropping systems could offer greater long-term returns.

We conducted an observational study to determine whether the combined effect of continuous cropping (i.e., intensification) and legume incorporation could further enhance SOC sequestration in the semi-arid region of the central U.S. We sampled fifteen long-term experimental plots and farmer fields under contrasting rotations of fallow-grain (no legumes) (FG-L), continuous grain without legumes (CG-L), and continuous grain with legume crops (CG+L) and compared the total SOC and N stocks to 1-m as well as crop yields, the degree of aggregation and amount of SOC and N in the surface (0-10 cm) soils as MAOM, POM [as both heavy (hPOM) and light (lPOM)], and dissolved organic matter (DOM) fractions. Additionally, we examined the long-term SOC responses to these management practices using the MEMS 1.0 model (Robertson et al., 2019) which simulates the effect of residue inputs and chemistry on the same SOC pools we measured.

2. Methods

Experimental Site and Design

Cropping Systems

We compared the most common dryland rotation of winter wheat followed by 14-months of fallow (fallow-grain without legumes, abbreviated FG-L) to grain rotations that increase the cropping intensity by growing a crop every year (Hansen et al., 2012) with and without the inclusion of a legume. The design sought to determine the potential for the intensification and the inclusion of a legume crop to enhance SOC accrual while maintaining or enhancing crop productivity. Farms with continuous rotations varied in the grain crops included, though were similar in that all planted a crop every year. Grain crops common to the continuous rotations were winter wheat, corn, proso millet, sunflower, and sorghum. We refer to the continuous grain rotations without a legume as CG-L. Finally, we refer to the continuous rotations that included legumes as a field pea as CG+L (‘*continuous grain with legume’*).

Fifteen dryland, no-till fields in eastern Colorado and western Nebraska were selected based on previous work by Rosenzweig *et al.* (2018) (Fig. 2.1). We sampled working farms and long-term agricultural research stations (ARS) with one or more of the three rotation types compared in this study: FG-L (n=5), CG-L (n=5), and CG+L (n=5). Sites were selected to have similar soil texture (loam or silt loam; Appendix Table 2.1), mean annual precipitation (MAP

395-457 mm $year^{-1}$), and mean annual temperature (MAT 8-11°C). Sites were clustered by climate as regional groups (Fig. 2.1). As the regional grouping was found to be meaningful for several soil C variables of interest (p-values 0.03 - 0.09), we included regional grouping as a random variable in our analyses. All fields sampled had been planted to winter wheat in fall 2017. We collected 5-year fertilization (Appendix Table 2.1) and management history from each field, and each site had been in its respective rotation for a minimum of eight years (Table 2.1).

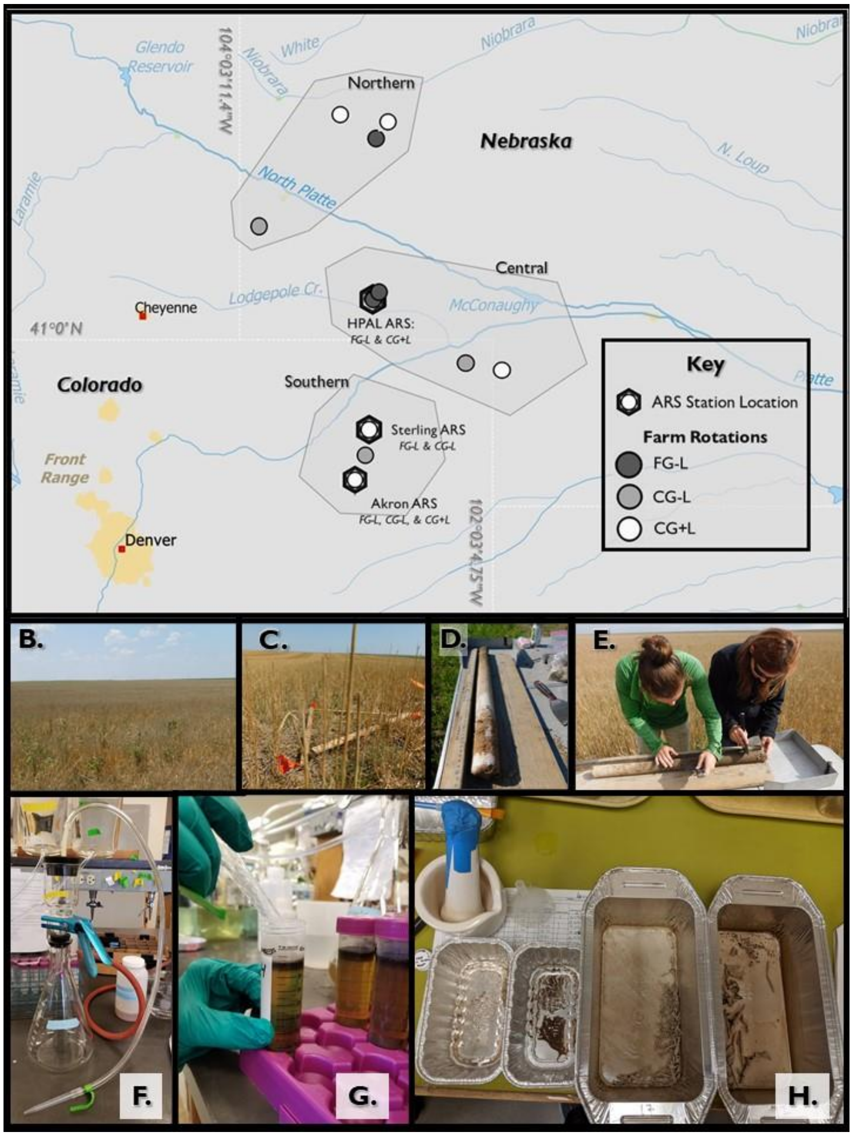

Figure 2.1 Site locations and experimental process. **A**. Gray polygon shapes indicate the three regions grouped by climate (Northern, Central, & Southern). Farms sampled are indicated by circles shaded by rotation where dark grey indicates FG-L (fallow-grain without legumes), light gray indicates CG-L (continuous grain without legumes), and white indicates CG+L (continuous grain with legume). Hexagons show locations of agricultural research stations (ARS) which contained multiple rotations in the study site. **B.** Typical field sampled displaying the standing wheat residue (2018). **C.** Triangle in place for collecting 3 soil cores for compositing into a single sample by depth increment as adapted from Spencer et al. (2011). **D.** Example soil core (0-1 m) after removed from the soil probe. Cores were placed on a cutting board and carefully cut into depth increments pictured in **E.** The density and size fractionation (**F-H**) consists of aspirating the light fraction particulate organic matter (POM) under vacuum pressure with the set-up as shown in **F**. A solution of sodium polytungstate at a density of 1.85 g mL^{-1} is used to float off the light fraction (**G.**). The remaining sediment is then wet-sieved and dried in pans (**H**) for subsequent analysis.

Soil Sampling

All soil samples were collected following wheat harvest in August 2018. To account for field variability while optimizing the number of cores to quantify SOC and N stocks, we subdivided each field into eight equally sized sub-sections, avoiding a 5 m border of each field to reduce potential edge-effect, and collected a sample from each section from a randomly selected (ArcGIS) point located by GPS. For the long-term experimental site in Akron, Colorado we collected a total of six replicate samples for each rotation due to the smaller size of each plot (0.08 ha) compared to other fields (average 50 ha, range 12-66 ha; ARS avg size 5 ha with range 0.08-20 ha). Each sample consisted of three soil cores collected at the vertices of an equilateral triangle with sides 1 m in length using a method adapted from Spencer et al. (2011) (Fig 2.1C). Briefly, each core (0-1 m) was collected with a truck-mounted, 3.8 cm diameter hydraulic probe (Giddings Machine Company, Inc., Windsor, Colorado). Upon extraction, cores were carefully checked for any sign of compaction and only non-compacted cores retained. Cores were divided into four depth increments (0-10, 10-30, 30-50, and 50-100 cm; Fig 2.2.1D & E) to assess potential management effects in surface soils (VandenBygaart et al., 2011) while still providing robust quantification of soil C and N stocks at depth (Holmes et al., 2015). The three samples per increment were composited and samples were stored at 4˚C until processed.

Laboratory Analyses

Soil Analyses for all sample depths (0-100 cm)

Laboratory analyses for gravimetric water content, bulk density, and total C and N were conducted as described by Conant *et al.* (2003). Briefly, each field-moist soil was weighed and passed through an 8-mm sieve. A sub-sample was then used for gravimetric water content by weighing before and after drying for 24 hours at 105˚C. Soils were further sieved to 2 mm, with root and rocks >2 mm separated, dried (60˚C), and weighed. We calculated bulk density based on the total sample mass minus soil water, roots, and rocks in the core volume calculated from the sample depth increment and soil core diameter. All analyses except water stable aggregates were performed using air-dried, 2-mm sieved soils. All bulk soil samples were analyzed for C and N content by dry combustion using a LECO Tru-Spec CN gas analyzer (LECO Corp., St. Joseph, MI, USA). We assessed which samples contained inorganic C by acid addition and quantified carbonates in bulk soil using the pressure transducer method (Sherrod et al., 2002). We estimated SOC as the difference of total soil C and inorganic C. We measured soil texture by hydrometer (Gee and Bauder, 1986) and pH using a 1:5 slurry of soil and deionized water.

Soil Analyses for surface soil (0-10 cm)

To assess the effect of management rotation on soil structure, we measured water-stable aggregates by wet-sieving. We used 40 g of 8-mm sieved soil and wet-sieved it through a series of three sieves (2000, 250, and 53 μm) to quantify the large macroaggregate (> 2000 μm), small macroaggregate (250-2000 μm), free microaggregate (53-250 μm), and free silt and clay (< 53 μm) size fractions. We sand corrected the > 53 μm fraction masses by measuring the proportion of sand (by mass) in 5 g of physically (glass beads) and chemically (0.5% sodium hexametaphosphate) dispersed soil (>53 μm) as described in Rosenzweig *et al.* (2018).

To quantify the C and N stocks in functionally distinct soil fractions, we used a fractionation scheme modified from Mosier et al. (2020) to obtain four fractions: dissolved organic matter (DOM), light particulate organic matter (lPOM), sand-sized, heavy particulate organic matter (hPOM), and silt and clay-sized, mineral-associated organic matter (MAOM). Briefly, we collected DOM by adding 35 mL of deionized water to 6 g of oven-dried 2-mm sieved soil sample and shaking gently for 15 min. Samples were centrifuged at 1069 gravitation for 30 min and the liquid decanted and frozen at -20°C until analyzed on a Shimadzu TOC-L/TNM-L Analyzer (Shimadzu Corporation, Kyoto, Japan) for organic C and N concentration. To disperse aggregates and separate the lPOM fraction we mixed the solid sample in a sodium polytungstate solution at a density of 1.85 g cm^{-3} and shook it with glass beads for 18 hours. After centrifuging, suspended soil particles were aspirated as lPOM (Fig. 2.1F & G), while the heavy fraction was further separated into hPOM (>53 μm) and MAOM (<53 μm) by wet-sieving (Fig 2.1H).

Total C and N was measured for the hPOM and MAOM fractions by dry combustion in the same way as the bulk soil with a LECO Tru-Spec CN gas analyzer (LECO Corp., St. Joseph, MI, USA). For hPOM and MAOM fractions where the 0-10 cm bulk soil had >0.09% inorganic C (IC) by mass (n=12), we acid-fumigated a sub-sample of the soil fraction with concentrated hydrochloric acid for 6-hours to remove IC (Harris et al., 2001). We quantified the C and N of these acidified fractions and the lPOM fractions, due to low sample mass, on an elemental analyzer-isotope ratio mass spectrometer (CN analyzer Costech ECS 4010 elemental analyzer Italy coupled to a ThermoFisher Delta V IRMS). Mass recovery after fractionation was 101% on average with a range of 95 to 108%. C recovery of the fractionation compared to bulk soil averaged 96% with a median value of 93% with a few (n=3) samples with large deviations (range 41 to 181%) from the typical value possibly due to high IC concentrations. N recovery averaged 97% with a median value of 94%.

Statistical Analysis

We performed all analyses using mixed linear effects models using R software (R version 4.0.2). To test the effect of rotation on measured soil characteristics, we used multiple mixed linear regressions followed by analysis of variance (ANOVA) and Tukey's honest significant difference (HSD) to test for pairwise comparison using the following R packages: *lme4* (Bates et al., 2015), *lmerTest* (Kuznetsova et al., 2017), *pbkrtest* (Halekoh and Højsgaard, 2014), *emmeans* (Lenth, 2018), and *car* (Fox and Weisberg, 2011). Prior to analysis, we performed an outlier test using Bonferroni's correction for multiple comparisons with a cutoff value of p = 0.05 and removed samples with Studentized residuals where $p < 0.0001$ (n=3 out of 453) as well as samples with missing data. These outliers were possibly due to the presence of fertilizer in the soil sampled as the N-content was ~30x above average values. To determine relevant covariates for the mixed linear effects models, we used corrected Akaike information criterion (AICc) values to select which covariates best-fit the data using the *MuMIn* package (Burnham & Anderson 2002) and selected the model with the lowest score. We allowed the model to select the possible covariates of percent sand and clay, soil pH, bulk density, MWD (for 0-10 cm), and included any covariates selected in the model to test the effect of rotation on the variable of interest (see Appendix Table 2.2 for summary of each model selected). For each mixed linear effects model, we placed variables on similar scales using the *standardize* (Eager, 2017) package and included sampling depth (where relevant), regional grouping (based on similar climate), and the sampling field as random effects to account for the variability these factors introduce. We considered $p \leq 0.1$ to be significant considering the variability among sites. The models followed the formula:

$$y_{ijk} = \mu_i + \alpha_{ijk} + B_j + C_k + \varepsilon_{ijk} \qquad \textit{(Eq. 1)}$$

where y is the variable of interest (i.e. SOC, SIC, soil N, MWD, or yield) for soil core i on field j at depth k, μ is the fixed effect of rotation (treatment), α is the fixed covariates based on AICc model selection (Appendix Table 2.2), B is the random effect of regional grouping (n=3), C is the random effect of depth where applicable (bulk SOC, SIC, and soil N), and ε is the variance on soil cores (*i,* n=6 or 8) collected from each field (*j,* n=15) at different depths (*k,* n=4).

Modeling the effect of rotation on soil organic carbon

We used the MEMS 1.0 soil C model (Robertson et al., 2019) to simulate the longer-term effects of the dryland rotations we studied on SOC stocks. Since SOC pools in MEMS 1.0 directly match the measured SOC fractions we quantified in this study, we were able to assess the accuracy of our simulation compared to field-measured values. While the full description of the model and equations can be found in Robertson *et al.* (2019), we summarize the most relevant model features here.

MEMS 1.0 combines the chemistry and C:N ratio of C inputs with the efficiency of microbial processing and the soil mineral matrix to simulate decomposition processes into measurable C pools (i.e., MAOM, hPOM, lPOM, DOM). In line with the framework for this study, the model is based on the hypothesis that N availability and the lignocellulose index (LCI; the ratio of the acid-insoluble plant C to the sum of the acid-insoluble plus acid-soluble) influences decomposition by altering microbial activity. In the model, daily litter C inputs are separated into structural or soluble (metabolic) litter pools (Cotrufo et al., 2015) with a carbon use efficiency (CUE) determined by the N and the LCI of the input material. The acid-soluble and insoluble fractions are estimated from literature-value measurements of plant tissue fiber and lignin via acid-detergent digest (Denef et al., 2008). Litter C pools can contribute directly to the POM fractions (lPOM and hPOM) via perturbation and fragmentation, and all C pools contribute to DOM through microbially-mediated decomposition at a rate and efficiency tied to the C-source elemental composition (chiefly C:N). The C entering the MAOM pool uses site values of soil pH and texture (sand content) to correspond with soil mineralogy and estimate binding affinity and MAOM saturation of C to the mineral matrix. DOM in the soil matrix can be microbially processed (respired), leached to deeper layers, or sorbed/desorbed to the mineral matrix as MAOM (Robertson et al., 2019).

Site-measured average values for soil pH, bulk density, percent sand and clay (Table 2.1) were used to initialize each field. MEMS 1.0 uses daily average temperatures to dictate a maximum rate of decay for each C pool. To obtain representative daily temperatures, we used weather data for the years 2015-2018 from on-site weather stations where available (the three ARS stations), or from the nearest weather station to the farm location from COAgMet ("CoAgMET"). These weather data coincide with the yield history data obtained from farm managers and thus allow conclusions to be drawn about change in SOC while ensuring the combined effect of weather on crop growth is accounted for. In cases where data were missing, we used previous day values or, for sequential missing days, we estimated average high and low temperatures based on the years with available data. Since investigating the effects of weather or climate change on soil C dynamics and crop productivity is beyond the scope of this work the same four years of weather and yield data were repeated for the duration of the simulation. This helps to ensure that any simulated directional change in SOC predicted by the model is due to rotation and management impacts, rather than temperature or rainfall.

We used literature values to estimate the C:N, acid-soluble fraction, and LCI of root and shoot material, and maximum rooting depth of each crop grown at each site (Appendix Table 2.3). Additionally, to estimate above and belowground C input based on crop yield, we used literature estimates of the harvest index and shoot:root ratio for each crop sown as follows:

$$C_{above} = Y*(1 - HI) / HI*[C] \qquad \textit{(Eq. 2)}$$

$$C_{below} = Y*(S:R * HI)*[C] \qquad \textit{(Eq. 3)}$$

where C_{above} and C_{below} are the aboveground and belowground biomass C, respectively, in g C m^{-2} yr^{-1}; *Y* is crop yield reported by farm managers in g C m^{-2} yr^{-1}, *HI* is the harvest index as a proportion total aboveground biomass (C_{above}) that is harvested as grain, [C] is the concentration of C in the residue or root tissue in percent, and S:R is the shoot to root ratio for a given crop (Bolinder et al., 2007, 1997).

The chemical litter characteristics for each rotation were approximated by performing a weighted average of shoot and root characteristics for each crop based on the S:R and the proportion of time each crop was part of a rotation as follows:

$$y_i = 1/n * (((1 - \mathrm{R{:}S}_k) * y_{\mathrm{root\text{-}k}}) + \mathrm{R{:}S}_k * y_{\mathrm{shoot\text{-}k}}) \qquad \textit{(Eq. 4)}$$

where y_i is the vegetation parameter (e.g., initial LCI of litter, litter %N, fraction of hot-water extractable and acid-insoluble material) for the i^{th} rotation, *n* is the proportion of time a crop is part of each rotation, R:S is the root to shoot ratio for the k^{th} crop and $y_{\mathrm{root\text{-}k}}$ and $y_{\mathrm{shoot\text{-}k}}$ are the literature values for the crop-specific parameters for root and shoot tissue respectively. This weighted-average approach was necessary since the model does not distinguish vegetation characteristics for above and belowground tissues or differentiate in the point of entry of the C input. These features are now present in the MEMS 2.0 ecosystem model (Zhang et al., 2021).

To estimate daily C inputs for each rotation, we selected typical planting and harvest dates based on common regional practice. For each day between seeding and harvest, the soil received a daily C input equal to the belowground biomass divided by the total number of days during the growing season. Aboveground biomass (C_{above}) was added all at once on the day of harvest to simulate the crop residue on the soil surface. Each field was averaged by rotation for each time-point with standard error (SE) reflecting the variance among fields (n=5) by rotation. These C inputs were used for the simulation in 4-year cycles based on yield data for each field from 2015-2018, in the same way that weather inputs were repeated across the same 4-year cycles. The consistent use of this 4-year cycle throughout the full model simulation ensures that C inputs and weather conditions are realistically coupled (as opposed to using long-term weather data which does not have any feedback to crop yield or C input) and helps to elucidate longer-term rotation/management impacts on SOC as opposed to any climatic impacts which are out of scope for this study.

The MEMS 1.0 was validated using data from European grasslands and forest soils (Robertson et al., 2019). Consequently, when the model was used to simulate cropland sites in Colorado, it was evident that parameter values for the decay rate and litter fragmentation of the POM (lPOM and hPOM) fractions did not represent the effects of management or semi-arid conditions, respectively. The original maximum rate of decay was based on a mean residence time (MRT) equivalent of 9.25 years for lPOM and 41.7 years for hPOM (del Galdo et al., 2003); we decreased the MRT to the equivalent of 3.7 years for lPOM and 33.4 years for hPOM similar to MRT determined by other studies in agricultural systems (Baisden et al., 2002; Beniston et al., 2014; Gaudinski et al., 2000). We also decreased the transfer of structural litter C into the lPOM pool (litter fragmentation) by 30% due to semi-arid, cropland systems having less active meso- and micro-fauna mixing the soil, especially during winter months. All changes to the original model parameters are within the standard deviation of the values used when parameterizing the original MEMS 1.0 model (Robertson et al., 2019). In order to verify acceptable model performance prior to the start of collected on-farm SOC data, we implemented a spin-up that initialized each field with the same SOC pool of 12 Mg C ha^{-1} and simulated a FG-L rotation for 80 years based on the average biomass input for the region (Table 2.2). We chose this starting value since it is in-between SOC stocks in the region found in grasslands which averaged 14 Mg C ha^{-1} for 0-10 cm (Gill et al., 1999) and those found in lands managed continuously as rangeland [9.96 $^{+}$/$_{-}$ 1.52 Mg C ha^{-1} (Li et al., 2017)]. The timeframe of the initialization seemed reasonable as the 1930s were a time when many farmers began conventional (shallower, less-frequent) tillage and when wheat-fallow rotations were promoted by agriculturalists at the time (Peterson et al., 2020). After the spin-up, the model simulated either a continuation of the same practice (FG-L) or conversion to an intensified rotation of CG-L or CG+L (model year 2010). This date corresponds to the date when many of the sites first implemented the intensified rotation with legumes (Table 2.1). The rotation conversion uses the C input values and litter characteristics of the new rotation to model changes to soil C in each pool. We simulated the new rotations out to 2080 to determine whether the mechanistic processes in the model indicate that these rotations may lead to long- or short-lived changes in SOC fractions.

To evaluate the goodness of fit (GOF) for how well the model predicted measured values for the year we sampled (2018), we performed a linear regression analysis (Smith and Rose, 1994; Piñeiro et al., 2008). In this test, modelled values with high GOF that vary consistently with measured data will have a slope equal to one; model bias can be assessed to the extent the y-intercept deviates from zero (Smith and Rose, 1994). We assessed the distribution of residual values and performed lack-of-fit and diagnostic assessments using the *olsrr* package (Hebbali, 2020). Prior to fitting the regression model, we removed six datapoints with high leverage as indicated by Cook's distance values > 1 (Cook 1977). Additionally, we calculated the root mean square error (RMSE) of the model residuals (the difference between measured and predicted values) for the fractions combined as well as individually to obtain an absolute measure of model fit (Wallach & Goffinet 1989). RMSE is in the same units as the predicted value and can be understood as the standard deviation of the unexplained variance where lower values indicate better model fit. As some fractions demonstrated differences between measured and modelled values that were not normally distributed, we used the non-parametric, paired Wilcoxon rank sum test to assess whether there were significant differences between measured and modelled SOC values (Bauer 1972). In this test, low *p*-values ($p<0.1$) indicate significant differences between measured and modelled values.

3. Results

Bulk soil C and N along the soil profile

Total SOC stocks across the 0-100 cm depth profile ranged from 38 to 61 Mg C ha^{-1} with a tendency for continuous rotations to have greater SOC (*ANOVA* $F=3.2$, $p=0.08$; Fig 2.2). Comparing depths helped detect differences across the treatment which were only significant for the CG+L in the top (0-10 cm) soil (Appendix Table 2.2 & 2.4; Fig 2.2).

Total soil N stocks (0-100 cm) averaged 6 to 7.3 Mg N ha^{-1} ($^{+}/_{-}$ 0.3 SE) with no differences by rotation (*ANOVA* $F=1.22$, $p=0.3$). Across rotations, the greatest stock of N was in the 10-30 cm depth which averaged ~ 0.5 Mg N ha^{-1} more than lower depths (*ANOVA* $F=59.6$, $p<0.001$; Fig 2.2).

Soil inorganic C (SIC) was present in many of the sites in this study and was quantified in order to calculate SOC from the total C. SIC was low or absent from surface soils (Fig 2.2), though it dominated soil C below 50 cm depth. SIC ranged from 5 to 90 Mg C ha^{-1} and was significantly higher under FG-L for most depths (average 53 Mg C ha^{-1} $^{+}/_{-}$ 13 SE; Appendix Table 2.2; Fig 2.2) from 0-100 cm; only in this rotation did SIC exceed SOC. FG-L consistently had 16-22 Mg C ha^{-1} more SIC than the continuous rotations, though there was high variability within and among fields (*ANOVA F=2.13, p=0.12*).

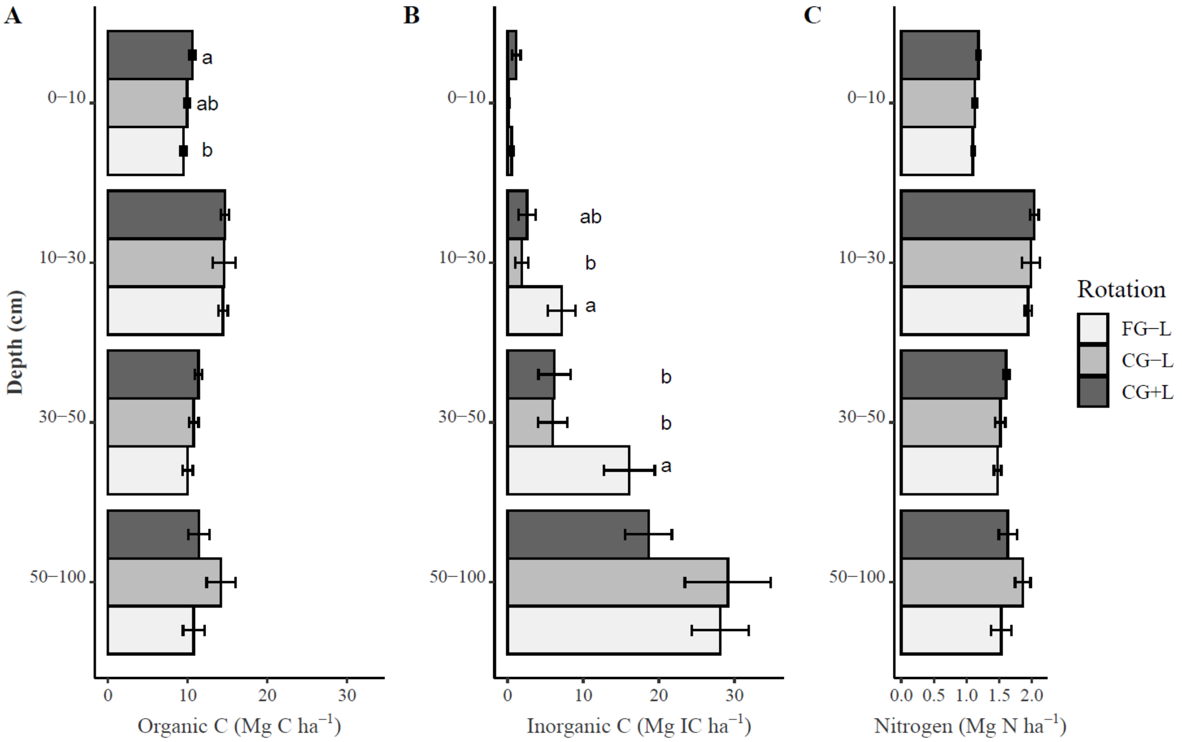

Figure 2.2 Bulk soil (A) organic carbon, (B) inorganic carbon, and (C) nitrogen stocks along the soil profile, for three crop rotations: FG-L = fallow-grain without legumes; CG-L = continuous grain without legumes; CG+L = continuous grain with legume. Error bars indicate standard error; n=5 fields per rotation. Letters indicate significant differences ($p \leq 0.1$) from FG-L based on *ANOVA* and least-squares means comparing treatment differences. For specific model results see Appendix Table 2.1.

Surface soil (0-10cm) properties

The mean weight diameter (MWD) of water-stable aggregates increased roughly 1.6-fold for both continuous rotations (CG-L and CG+L) compared to the FG-L (Fig. 2.3, *ANOVA F=4.64, p=0.03*); the increased aggregate size in these rotations was mainly due to them having on average 137% greater proportion of large macroaggregates (>2,000 µm; 15% in continuous rotations *vs.* 6% in FG-L). Aggregates were 496 µm greater on average (*t=3.0, p=0.03*) in the CG-L than the FG-L rotation, though similar for CG+L (357 µm greater than FG-L; t=2.1, p=0.13). The proportion of free microaggregates (53 – 250 μm) was ~5% higher in the FG-L rotation than both CG-L (*t=2.4, p=0.05*) and CG+L (*t=2.7, p=0.02*). We did not investigate the amount of microaggregates contained within macroaggregates.

The rotation with legumes (CG+L) had greater SOC (and N) stocks both as measured in the bulk soil as well as the sum of the SOM fractions C (and N) from 0-10 cm than FG-L (Fig. 2.2 & 2.4; Appendix Table 2.4). Overall CG+L had 12% greater bulk SOC compared to FG-L (*t=2.4, p=0.*08), though no difference in bulk soil N (*t=1.7, p=0.25;* Fig 2.2). These differences were more apparent comparing SOM fractions.

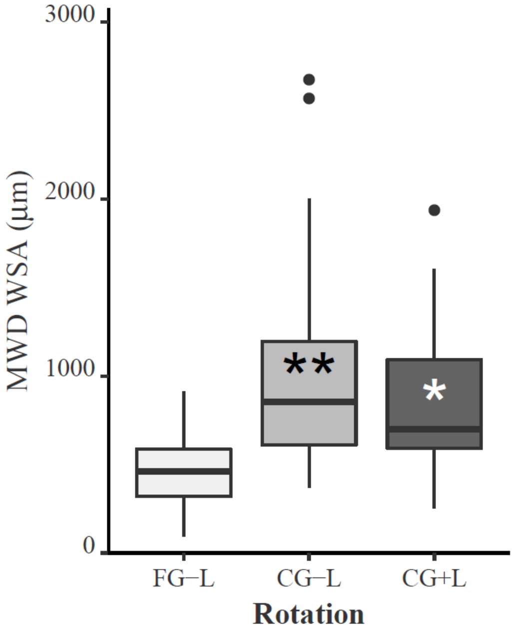

Figure 2.3 Boxplot of the mean weight diameter (MWD) of water stable aggregates in micrometers by rotation. Boxes represent the data that falls within the 25-75% quantiles with median values represented as the central line. Whiskers represent 1.5 times the interquartile range of the 25th and 75th percentiles while dots indicate values outside of this interval (outliers). Rotations are as described in Table 2.1. Double asterisks (**) indicate $p \leq 0.05$; single asterisks (*) indicate $p \leq 0.1$ based on *ANOVA* and least-squares means comparing treatment differences from FG-L; for specific model results see Appendix Table 2.1.

Table 2.1. Site, soil and management characteristics for each field sampled in this study. All fields were located in Eastern Colorado or Western Nebraska (Figure 1) at a privately managed farm ('Farm') or agricultural research station (ARS). Fertilizer (Fert) application rates are the sum of the N-application over four years. Rotation (rot.) categories were as follows: FG-L = fallow-grain without legumes (*wheat fallow)*; CG-L =continuous grain without legumes; CG+L = continuous grain with legumes. Some farms deviated slightly from these exact rotations (see 'Actual Rot.'). Letters represent crops planted in a given year as follows: W=Wheat; C=Corn; M=Millet; P=Pea; Su=Sunflower; So=Sorghum. MAP=Mean annual precipitation; MAT=Mean annual temperature. SIC= soil inorganic carbon (C); SOC = soil organic C; N= nitrogen summed for the soil profile (0-100 cm); *n.d.* indicates no data available. Values in parentheses represent standard deviation from mean for a total (tot) number (n) of samples (6-8 soil cores x 4 depth increments). Some soil cores had a maximum depth less than 100 cm due to rocks and hard pan, which is why not all soil cores have the same number of samples. **For many sites, the years under rotation reflect the start of a research experiment or year of land acquisition by the current land manager. For research stations, prior history was typically conversion from a conventionally-tilled wheat-fallow system. Prior history for farms was unable to be determined, though year of acquisition tended to also be the last year land managers were aware of a field being tilled and adopted current management.*

Rot.	**Farm or ARS**	**Actual Rot.**	**MAP** (mm)	**MAT** (°C)	***n***	**Bulk Density** (g cm^{-1})	**Clay** (g kg^{-1})	**pH**	**Tot SIC** (Mg C ha^{-1})	**Tot SOC** (Mg C ha^{-1})	**Tot N** (Mg C ha^{-1})	**4-yr Yield** (kg ha^{-1})	**Sum 4-yr Fert-N** (kg N ha^{-1})	**Yr began rot.***
FG-L	Farm	WFWF	457	9	32	0.88 (0.08)	240 (60)	7.60 (0.8)	18 (40)	57 (24)	7.2 (2.2)	2,001	135	1986
	Farm	WFWF	395	8	32	1.01 (0.07)	250 (80)	8.25 (0.6)	98 (77)	41 (18)	5.7 (1.5)	1,520	150	1984
	Akron ARS	WFWF	405	10	24	0.92 (0.04)	260 (80)	7.64 (1.2)	43 (23)	38 (10)	6.1 (1.3)	2,232	112	1990
	HPAL ARS	WFWF	457	9	32	0.90 (0.06)	240 (40)	7.99 (0.8)	29 (30)	44 (13)	6.0 (1.3)	1,616	135	1972
	Sterling ARS	WFWF	440	9	32	0.94 (0.06)	270 (50)	8.21 (0.8)	75 (44)	42 (11)	5.3 (1.3)	1,036	90	1985
CG-L	Farm	WWWW	405	10	32	0.93 (0.06)	300 (90)	7.99 0.9)	64 (63)	49 (12)	6.4 (1.2)	1,964	34	2005
	Farm	WMSuC	430	10	31	0.90 (0.06)	260 (70)	7.64 (0.8)	13 (17)	61 (29)	6.7 (1.4)	3,615	154	2009
	Farm	WMSuC	411	9	31	0.93 (0.07)	170 (40)	7.74 (0.7)	5 (8)	43 (11)	6.2 (1.3)	3,319	112	2006
	Akron ARS	WCMW	405	10	24	0.91 (0.03)	270 (90)	7.68 (1.0)	39 (14)	52 (28)	7.5 (2.8)	2,832	163	1990
	Sterling ARS	WCMSo	440	9	28	0.93 (0.04)	280 (70)	8.13 (0.9)	64 (58)	44 (12)	5.7 (1.7)	1,707	45	1985

CG+L	Farm	WCPM	420	11	32	0.94 (0.05)	270 (70)	7.73 (0.8)	19 (35)	56 (14)	6.7 (1.5)	2,967	141	2012
	Farm	WCMP	395	8	32	0.94 (0.09)	230 (60)	7.55 (0.9)	18 (35)	47 (15)	6.3 (1.8)	*n.d.*	*n.d.*	2010
	Farm	WCPW	395	8	31	1.02 (0.08)	210 (60)	7.70 (0.9)	28 (39)	41 (11)	5.7 (1.4)	3,040	78	2010
	Akron ARS	WCMP	405	10	24	0.93 (0.05)	280 (80)	7.72 (1.1)	40 (28)	44 (13)	6.5 (1.4)	1,942	95	1990
	HPAL ARS	WPMSu	457	9	29	0.88 (0.10)	230 (50)	7.91 (0.8)	44 (47)	52 (17)	7.3 (1.6)	2,223	123	2010

Table 2.2. Estimated total annual above and belowground soil C inputs by rotation based on yield and biomass data from each field. Rotations are as described in Table 2.1.

Rotation	**Above Biomass Input** ($g\ C\ m^{-2}\ yr^{-1}$)	**Belowground Biomass Input** ($g\ C\ m^{-2}\ yr^{-1}$)	**Percent Difference from FG-L**
FG-L	205	64	
CG-L	285	69	+32%
CG+L	288	63	+31%

The largest difference in SOM fraction C and N stocks were in the MAOM, with higher MAOM C and N in CG+L than FG-L (*t=2.9, p=0.01* for C; *t=2.5, p=0.03* for N*;* Appendix Table 2.4; Fig. 2.4). Both continuous rotations had 0.3 Mg C ha^{-1} ($^{+}/_{-}$ 0.05 SE) greater stocks as hPOM than FG-L (*t=2.7, p=0.03* CG+L; *t=2.3, p=0.07* CG-L), though only CG+L also had greater N in this fraction (*t=2.1, p=0.1).* Though a relatively small and transient pool, the DOM C and N was only higher in CG+L rotation compared to FG-L by ~0.05 Mg C ha^{-1} ($^{+}/_{-}$ 0.01 *SE*; *t=2.5, p=0.04*) and 0.003 Mg N ha^{-1} ($^{+}/_{-}$ 0.002 SE; *t=2.0, p=0.1*). There were no differences in the light-fraction (lPOM) among rotations for C (*F=1.5, p=0.2)* or N (*F=1.7, p=0.2*).

Crop Yields

As all of the fields we sampled were under wheat cultivation the summer of 2018, we are able to compare crop yield directly for that year (Fig. 2.5). FG-L rotations had 30% higher wheat yields than the continuous rotations in 2018 (*F=1.3, p=0.3*) though there was high variability, partially due to hail at one sampling site. Considering average annual yield over a four-year crop rotation cycle, FG-L underperformed the continuous rotations by an average of 930-1030 kg ha^{-1} ($^{+}/_{-}$ 430 SE; *ANOVA F=4.86, p=0.04*) since as FG-L rotations harvest a crop only twice in a four-year cycle. Additionally, FG-L fields had higher rates of N-fertilizer applications on average (124 kg N ha^{-1} over 4 years *vs*. 101 and 112 for CG-L and CG+L respectively) (Table 2.1).

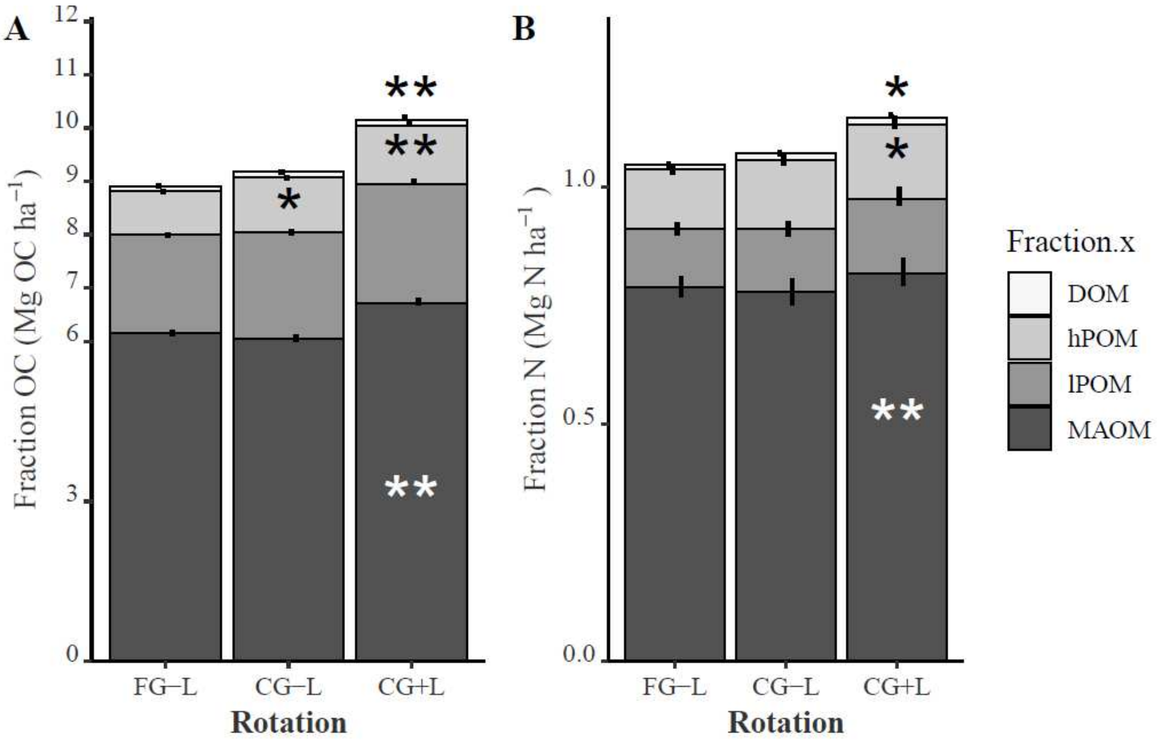

Figure 2.4 Soil organic carbon (OC) and nitrogen (N) stocks for the four soil organic matter fraction by rotation. Rotations are as described in Table 2.1. MAOM=mineral-associated organic matter (OM); lPOM=light-fraction particulate OM; hPOM = heavy particulate OM; DOM=dissolved OM. Error bars indicate standard error; n=5 fields per rotation. Double asterisks (**) indicate $p \leq 0.05$; single asterisk (*) indicates $p \leq 0.1$ based on ANOVA and least-squares means comparing treatment differences from FG-L. For specific model results see Appendix Table 2.3.

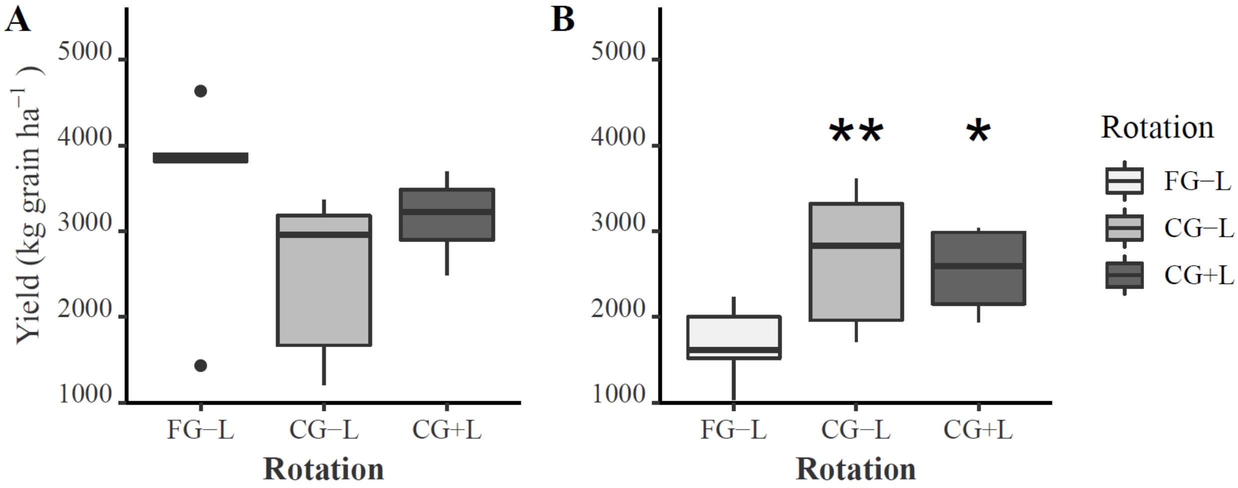

Figure 2.5 Crop yield in (A) 2018 and (B) 4- year average across rotations in kilograms grain per hectare. Boxes represent the data that falls within the 25-75% quantiles with median values represented as the central line. Whiskers represent 1.5 times the interquartile range of the 25th and 75th percentiles while dots indicate values outside of this interval (outliers). Double asterisks (**) indicate $p \leq 0.05$; single asterisk (*) indicates $p \leq 0.1$ based on ANOVA and least-squares means comparing treatment differences from FG-L. For specific model results see Appendix Table 2.1. Rotations are as described in Table 2.1.

Future soil C scenarios

The MEMS 1.0 model represented the distribution of SOC across soil fractions well, having a mean difference between measured and modeled values of 0.41 Mg C ha^{-1} (*RMSE*; Fig. 2.6; Appendix Table 2.5). The model had a tendency to overestimate SOC as evidenced from slope and intercept of a linear regression of the measured and modelled values (Fig. 2.6):

$$\hat{y}_i = 16.1 + y_i * 0.86 + \varepsilon \qquad (Eq.\ 5)$$

where $\hat{y}$ are the measured SOC values for the i^{th} field, y is the modelled SOC values, and ε is the error term ($^{+}/_{-}$ 0.64). A perfect model simulation would have a slope equivalent to one and a *y*-intercept equal to zero. This overestimation was driven, in part, by values from the Akron ARS site, where measured values were consistently below model predictions. Removing this site from the analysis decreases the RMSE for the total C, MAOM, and hPOM fractions and improves the R2 of the regression by 30% (Appendix Table 2.4). The model did not perform well for individual soil fractions of DOM and hPOM (Appendix Table 2.5) as the paired Wilcoxon Rank test indicated both values came from the different underlying distributions. There were no obvious model biases by rotation type (Fig. 2.6, Appendix Fig. 2.1),

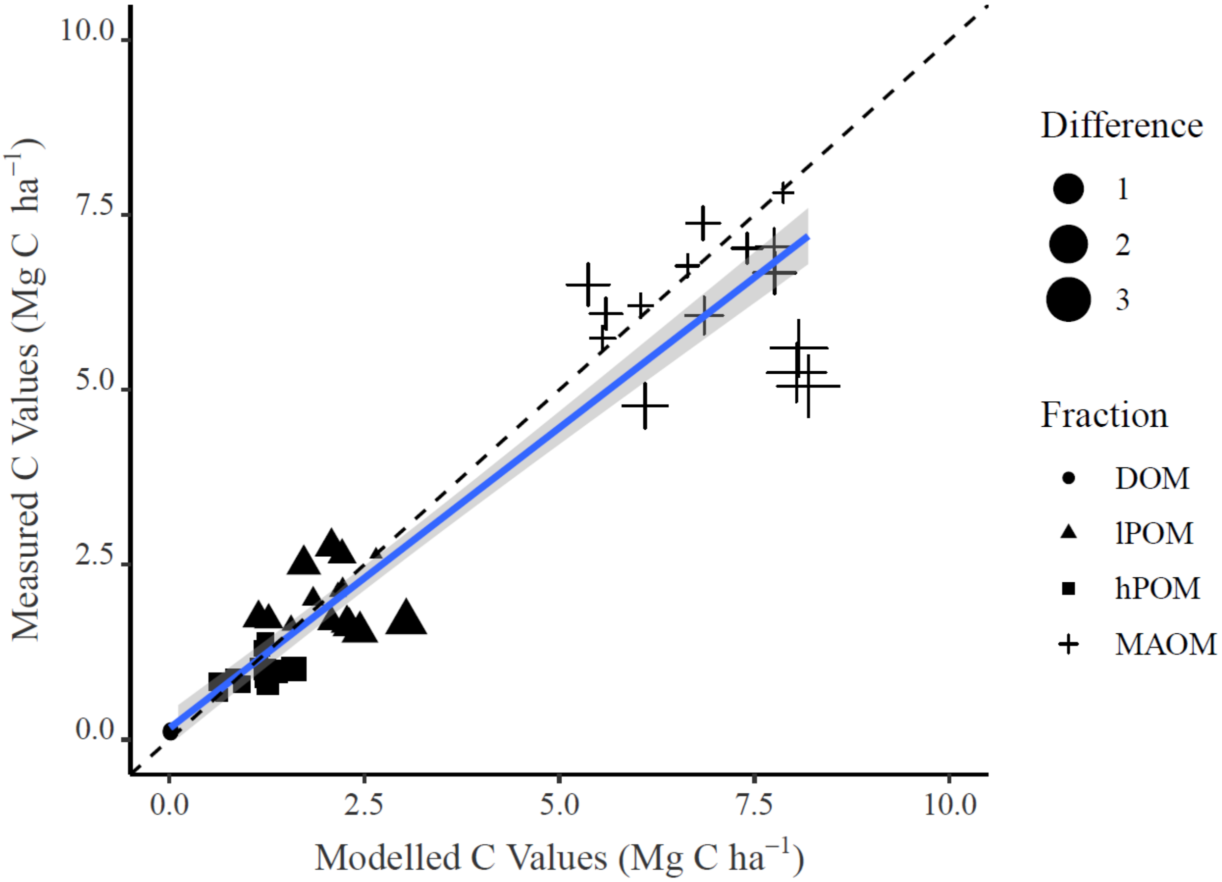

Figure 2.6 Goodness of Fit of measured and modelled values in 2018 by soil fraction and field sampled. The size of each shape ('Difference') represents the absolute value of magnitude of difference between measured and modeled values for each field. Correlation coefficient= 0.96, $R^2 = 0.96$. Each shape represents the SOC fraction field-sampled and modeled in this study (n=15); fractions are as described in Figure 2.4. Units are in Mg C ha^{-1}. The dashed line represents a 1:1 line of modelled to measured values; the solid line represents the best-fit regression among data points; the grey area around the regressed line (Eq 5) represents the 95% confidence interval. See Appendix Table 4 for the regression and model-fit for each fraction.

We initialized all sites under the same rotation of FG-L for 80 years, and this resulted in a loss of SOC of roughly 2 Mg C ha^{-1} (mean C at end of initialization 9.9 Mg C ha^{-1} +/- 0.8). After implementing the rotation change (CG-L & CG+L), soil fraction C reached equilibrium within 20 years for POM (sum of hPOM and lPOM) and DOM fractions (Fig. 2.7) and increased in all fractions The MAOM-C fraction increased only slightly in CG-L rotation at a rate of +4 kg C ha^{-1} yr^{-1} (compared to an average change of -9 kg C ha^{-1} yr^{-1} under FG-L) but the MAOM-C in the CG+L rotation increased three times as fast (15 kg C ha^{-1} yr^{-1}) (Fig. 2.7B).

The continuous rotations had similar input quantities (Table 2.2) and similar increases in SOC in the POM fractions. Twenty years after implementing the continuous rotation, CG-L and CG+L had similar total SOC (~11 Mg C ha^{-1} yr^{-1} CG+L & CG-L vs. 9 Mg C ha^{-1} yr^{-1} FG-L), though a greater proportion of the total SOC under CG+L was as MAOM-C (71% in CG+L vs. 66% CG-L). The CG+L rotation differed from the CG-L and FG-L by continuing to increase MAOM-C for the duration of the simulation while CG-L reached steady-state conditions 32 years after introducing the rotation change. By 2100, total C in CG+L exceeded FG-L by 3.7 Mg C ha^{-1} yr^{-1} and exceeded CG-L by 1 Mg C ha^{-1} yr^{-1}, recovering the previously lost soil C. Overall, CG+L gained SOC five times faster than CG-L (Fig. 2.7).

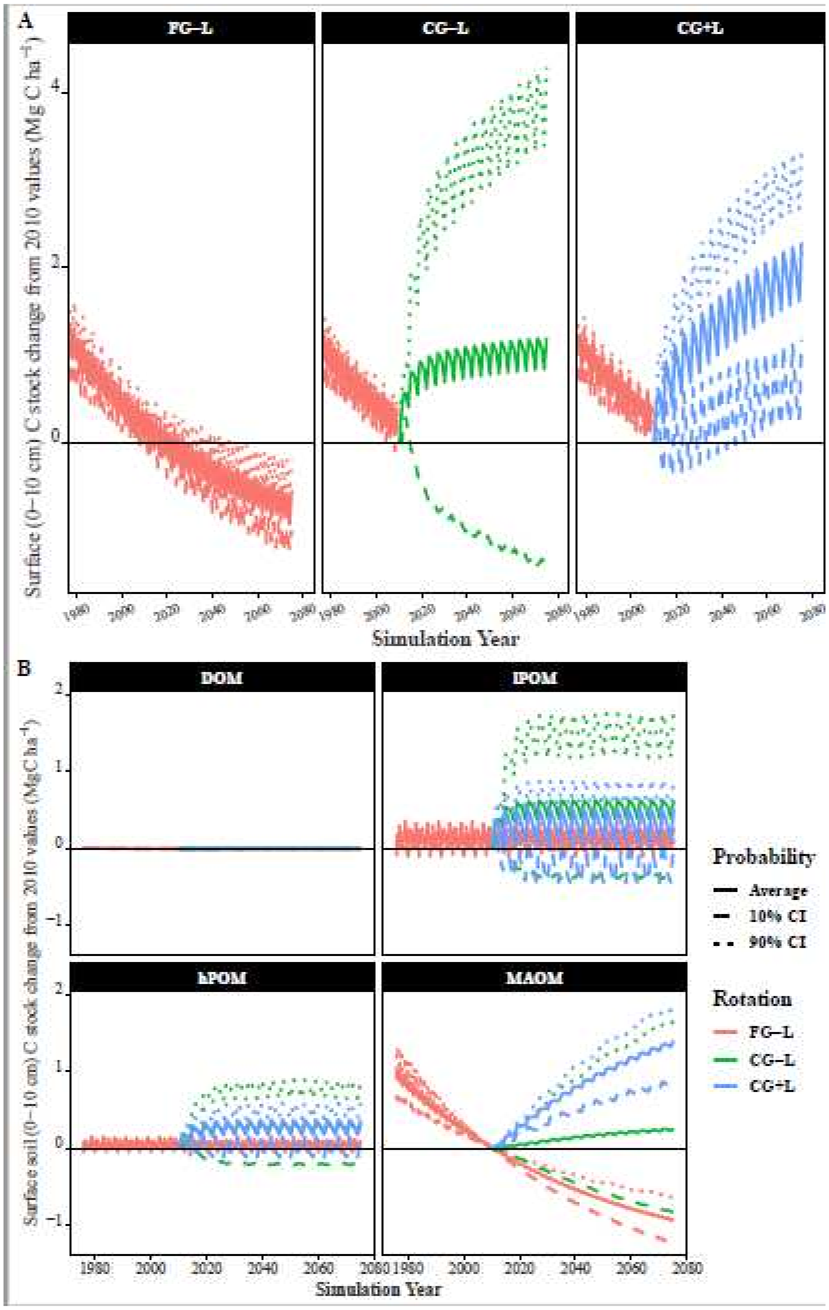

Figure 2.7 Dryland MEMS 1.0 simulations of soil C stocks for the 0-10 cm depth under wheat-fallow rotation for 80 years, followed by three scenarios representing the measured systems (Table 1): continued FG-L (red), intensification (CG-L - blue), and intensification with legume inclusion (CG+L - green). A. Depicts change from 2010 value of the total carbon under each scenario. **B**. Depicts the soil C pools from the simulation that were measured in this study. Fractions are as described in Figure 4. Units are in Mg C ha^{-1}, (n=5 per rotation); shaded area represents the 90% confidence interval around the mean (bold, solid line) of the fields in this study by rotation.

4. Discussion

The purpose of this study was to assess whether the form of inputs used to intensify and diversify cereal cropping systems can address the soil C dilemma (Janzen, 2006) by influencing the productivity and form of SOC and N accrual compared to conventional practices of wheat-fallow. Management effects were detectable in the surface (0-10 cm) soils and were more pronounced through examining the SOM fractions. Both the SOM fraction measurements and modelled values for SOC (0-10 cm) supported our hypotheses that incorporating legumes into a continuous grain rotation enhances SOC through increasing MAOM, while maintaining or even enhancing POM stocks and overall crop yields. These findings support the significance of plant input amount and chemistry in influencing the efficiency of C-stabilization in soils and the potential for management practices that simultaneously increase input quantity and N-content (quality) to restore SOC to soils in dryland, semi-arid agroecosystems, effectively solving the soil C dilemma.

Inconclusive changes to bulk soil C and N for 0-100 cm profile

Many studies of management effects on soil C focus primarily on the top 20 cm as that is the region of primary soil C inputs from roots and residue (Conceição et al., 2013) and is the most responsive to management effects such as fallow (VandenBygaart et al., 2011). In this study, we examined the soil profile to 100 cm as up to 50% of SOC in agricultural lands could lie in sub-soils (20-200 cm; Buyanovsky and Wagner, 1986), and there's potential for C redistribution and changes to SOC and SIC dynamics at deeper depths that are poorly understood (Zamanian et al., 2016). While there was a tendency for continuous rotations to have higher SOC and soil N than FG-L, we could not conclusively detect changes to SOC stocks (Fig 2.2). The lack of significant differences across the full soil profile in our study was not due to inverse responses in top and subsoil layers (Fig 2.2). As deeper soils tend to have greater variability in SOC, larger sampling efforts are needed to detect significant management effects along the full soil profile (Kravchenko and Robertson, 2011).

Management effects in no-till systems typically are most pronounced in the soil surface as it is the most dynamic portion of the soil profile (Six et al., 2000; Wilpiszeski et al., 2019). This study was consistent with that trend (Fig 2.2, 2.4). We observed significant differences only in the CG+L rotation compared to FG-L and not in both continuous rotations, suggesting that factors beyond cropping frequency and estimated soil C input (Table 2.2) influence SOC and N stocks since these were similar for the continuous rotations in this study. Further, these findings suggest that greater retention of N (from legumes) may reduce the priming effect of native SOC as has been demonstrated in a mechanistic study (Bernal et al., 2016) leading to greater microbial efficiency (Kallenbach et al., 2015) and overall greater SOC and soil N stocks. In contrast, another study in the same region found continuous rotations increased SOC stock by 17% (0-10 cm) and 12% (10-20 cm) (Rosenzweig et al., 2018), suggesting we may have also observed increased SOC in CG-L rotations compared to FG-L with a larger dataset.

We also observed patterns that could indicate an altered hydrologic regime in the continuous rotations. Surprisingly, the FG-L rotation had 10% and 20% more total soil C than CG-L and CG+L rotations respectively due to the large amount of SIC at depth that comprised the majority (54%) of total soil C in those fields (Fig. 2.2; Appendix Table 2). Other studies in semi-arid regions have similarly observed carbonates (IC) accumulate at depth especially under wheat-fallow rotations (Denef et al., 2008; Plaza-Bonilla et al., 2015). Cultivation, particularly summer fallow, likely increases SIC at depth in relation to the root biomass where decreased root density promotes deeper water infiltration and evaporation and thus SIC accrual (Powlson et al., 2011).

Crop yields and fertilizer efficiency higher under continuous rotations

Annualized crop yields were enhanced under both continuous rotations (Fig. 2.5) supporting well-established evidence from the region that eliminating the summer fallow can increase overall system productivity (Peterson et al., 2020; Rosenzweig and Schipanski, 2019). When considered over the full rotation cycle, annual cropping increases productivity likely due to the enhanced water-use-efficiency from increased aggregation and infiltration (Peterson et al., 2020; Rosenzweig et al., 2018) and increased cropping during periods with higher precipitation and potential evapotranspiration (Peterson et al., 1996). CG+L rotations may also have greater water-use-efficiency compared to CG-L due to the shorter growing season of peas, reducing seasonal water consumption and leaving more available for subsequent crops. Continuous rotations were also more efficient with fertilizer N, producing 83% more grain per unit fertilizer N than FG-L (Table 2.1). The similar productivity (Fig. 2.5B) and fertilizer efficiency of both CG-L and CG+L rotations demonstrate that incorporating legumes did not compromise productivity which can be a concern with the lower biomass and yields of legumes compared to cereal crops (Jensen et al., 2012). A recent study in the Central Great Plains found that the period from 2008-2016 marked the first time that wheat-fallow was not the dominant cropping system as intensified rotations are increasingly replacing summer fallow (Rosenzweig and Schipanski, 2019).

Top Soil Properties (0-10 cm)

Continuous cropping promotes aggregate stability but does not explain changes to SOC

Both continuous rotations (CG-L and CG+L) had a higher proportion of macroaggregates compared to FG-L (Fig. 2.3) with a 67% increase in average size, supporting previous findings that found aggregate size increases with continuous cropping systems (Rosenzweig et al., 2018). Continuous rotations may promote increased aggregate size since a plant canopy and increased root density protect soil during the majority of annual precipitation in May and June (Peterson et al., 2020).

Macroaggregates (>2,000 μm) are more responsive to management practices such as tillage and residue retention than microaggregates (53 - 250 μm). Since they are dynamic structures (Six et al., 2000) and tend to contain higher concentrations of labile C (Trivedi et al., 2015) that readily decomposes, macroaggregates may ultimately be poor protectors of SOC. This may explain how, despite similar MWD of WSA in both continuous rotations (Fig. 2.3), only CG+L contained higher amounts of SOC (Fig. 2.2). Other studies have found, however, that macroaggregates may promote the accumulation of MAOM and protection of POM within microaggregates (King et al., 2019). There may be a dual interaction of SOC and aggregation where increases to SOC can enhance aggregation and *vice versa* (Powlson et al., 2011). Differences in stoichiometry of soil input between CG-L and CG+L may explain the lack of interaction in this study. Fertilization in agricultural soils may lead to SOC loss without sufficient residue return (Fan et al., 2020), while legumes may promote soil C within mega-aggregate structures (Tiemann et al., 2015). The similarly large proportion of macroaggregates observed under CG-L and CG+L may mask more subtle differences in SOC dynamics due to the different source of N in these systems.

Rotations with legumes enhance MAOM and hPOM, grain-only rotations affect hPOM fraction

Examining the C and N stocks distribution among SOM fractions in conjunction with the yield data and model simulation enabled us to evaluate whether integrating legumes into dryland grain systems ‘solved’ the soil C dilemma as well as to understand how management is influencing surface SOM. The majority (77-97%) of C added to soil is mineralized in the short-term (<42 years) (Castellano et al., 2015), and many factors are known to affect the likelihood and degree of long-term SOC stabilization such as the proportion of structural *vs*. metabolic (soluble) inputs (Soong et al., 2015) point of entry (Sokol et al., 2019), and underlying soil texture (Zeller & Dambrine 2011) and minerology (Singh et al., 2016). Of these, we explored how the input and content may influence the distribution of SOC in soil fractions. We hypothesized that increased inputs from continuous cropping would likely contribute primarily to the POM fractions (lPOM and hPOM) as grain crop residues typically have a high C:N ($\geq$80), largely structural components, deposited mainly aboveground so that, when decomposed, would result in the majority of C to be lost as CO_2 while provisioning N to the microbial community and plant roots. Plant root exudates, and the soluble proportion of aboveground residue (2-16% Soong et al., 2015) and root tissues from both grain and legume sources are likely to contribute preferentially to MAOM and to a lesser extent to hPOM (Cotrufo and Lavallee, *2021*) as has been found in previous studies (Samson et al., 2020). Legume residue with its lower C:N and higher soluble C and N content could decompose more efficiently (Cotrufo et al., 2013; Kallenbach et al., 2015), resulting in a greater proportion of C that could readily adhere to mineral surfaces as MAOM (Angst et al., 2021; Kleber et al., 2015). Simultaneously, some of the N released during microbial mineralization of these low C:N inputs would be used to support plant growth. Legumes may also have a greater proportion of root exudates and belowground C allocation compared to cereal crops (Bolinder et al., 2007), increasing the proportion of legume-derived C likely converted to SOC as several studies have revealed that root litter and exudate C are disproportionately retained as SOC (Rasse et al., 2005; Villarino et al., 2021) compared with surface litter, and that root C is more likely to be protected from decomposition within aggregates than shoot material (Baumert et al., 2018; Fulton-Smith & Cotrufo 2019).

The fractions of C and N stocks in MAOM and POM we observed in the continuous rotations (Fig. 2.4) and the predicted response of SOC to management from MEMS 1.0 (Fig. 2.7) support these hypotheses for the surface soil. Rotations with legumes (CG+L) had increased SOC equally from MAOM and POM fractions (MAOM: 0.6 Mg C ha^{-1} $^{+}/_{-}$ 0.2 SE; POM: 0.7 Mg C ha^{-1} $^{+}/_{-}$ 1.1 SE), while CG-L rotations had only greater C in the POM fractions (0.3 Mg C ha^{-1} $^{+}/_{-}$ 0.9 SE) (Fig. 2.4) compared to FG-L. As both continuous rotations had similar average yields (Fig. 2.5), estimates of soil C inputs based on yield (Table 2.2), N fertilization (Table 2.1), and aggregate stability (Fig. 2.3) with no differences in soil texture or climate, the main difference among these practices is the inclusion of legumes. That N increased only in the CG+L rotation where C also increased (Appendix Table 2.4) implies this rotation may promote enhanced microbial CUE per unit N (Cotrufo et al., 2013; Mooshammer et al., 2014). The model simulation corroborates this possible mechanism as it demonstrated that increasing inputs from a FG-L rotation would lead to greater soil C primarily as POM, but that MAOM would only continue to accumulate when litter inputs have a higher N-content (Fig. 2.7B).

There’s a strong precedent demonstrating that legumes may aid efficient N cycling within the soil compared to cereal crops. Studies of pulse crops have found that peas in particular tend to confer the greatest N benefit (11-14 kg N ha^{-1}) to subsequent crops compared to other legumes such as chickpea, lentil, common bean, or soy (Przednowek et al., 2004). Legume residues, even excluding potential root contributions, confer greater soil N than residue from cereals (Lupwayi and Kennedy 2007) and their inputs are preferentially retained as SOM (Drinkwater et al., 1998; Gregorich, et al., 2001). Many studies have documented how increasing N availability can lead to greater C accrual (Averill and Waring, 2018; Yue et al., 2016). Our study suggests that the form of that N could also be significant as N-fertilization tended to be slightly higher in the CG-L rotations on account of fertilizer being applied each year (farms and ARS in this study did not apply fertilizer to the pea crop), yet SOC was only significantly greater when legumes were incorporated. Many studies support our findings of intensification leading to increased soil structure and SOC (Bowles et al., 2020; Oldfield et al., 2019; Rosenzweig et al., 2018), and that legume crops may confer additional C sequestration benefits of up to 15-21 Mg C ha^{-1} (Jensen et al., 2012; Meena et al., 2018). Legumes have not always proven to enhance SOC, however, as multiple studies have found legumes had no overall effect likely due to their reduced biomass compared to grain crops (King and Blesh, 2018; Sainju et al., 2007).

Intensification with legumes enhances both short and long-term SOC accrual

Discussion of SOC accrual is often reasonably tied to the question of persistence and turnover of SOC within the soil (Torn et al., 2009). The fractions we measured in this study have different age distributions based on ^{14}C dating with lPOM fractions having the youngest C and MAOM the oldest (Cotrufo and Lavallee, *in press*; Lawrence et al., 2020). While a relatively small proportion of the MAOM fraction is dynamic with actively exchanging organo-materials, most of the C in MAOM is stabilized by the mineral association leading to greater SOC persistence through MAOM (Kleber et al., 2015). Thus, we argue that the farms (CG+L) that had higher MAOM C are likely accruing persistent SOC as this form of C is less subject to mineralization losses relative to other fractions.

The hPOM pool reflects partially decomposed plant and possibly microbial structures (Lavallee et al., 2020) that some have argued is more similar to MAOM than to lPOM (Samson et al., 2020) which may explain, in part, how this fraction was higher in the CG+L rotation as both C and N (Appendix Table 2.4) where MAOM was also a larger pool. We expected to see greater stocks of both hPOM and lPOM with more frequent and diverse plant growth as has been demonstrated by previous studies (Crow et al., 2009; Li et al., 2020). As hypothesized, we observed larger hPOM on the order of ~0.3 Mg C ha^{-1} ($^{+}/_{-}$ 0.05 SE) in both CG-L and CG+L rotations relative to FG-L (Appendix Table 2.4). Surprisingly, we did not observe differences to the lPOM fraction in continuous rotations. lPOM consists primarily of fragmented plant materials subject to rapid decomposition (<10 yrs) (Lavallee et al., 2020). As we sampled all of the fields shortly following wheat-harvest, this fraction was likely a reflection of the most recent crop since this fraction does not accumulate in agricultural systems. Fertilizer has also been found to accelerate decomposition of POM (Neff et al., 2002), potentially further explaining the small lPOM accumulation despite increased input in the continuous rotations.

Overall, our observed larger SOC stocks in the CG+L rotations are in line with broad estimates of the potential for management practices such as no-till and crop rotations to enhance C accrual rates which range from 0.3-0.6 Mg C ha^{-1} yr^{-1} (Minasny et al., 2017). As this study was conducted at a single time point and the fields involved under the various rotations for varying lengths of time (range of 8 - 30+ yrs; Table 2.1), it is not possible to estimate a SOC accumulation rate or loss from this study. The ~1 Mg C ha^{-1} (0-10 cm) greater SOC with legume cultivation (CG+L) measured in this study seems plausible given the rates observed in previous studies which have averaged 0.57 Mg C ha^{-1} yr^{-1} in an alternating corn-legume rotation (Meena et al., 2018).

Compared to FG-L, fields with CG+L rotations had greater SOC and soil N in both MAOM and hPOM fractions while sustaining high rates of productivity (Fig. 2.3 and 2.4; Appendix Table 2.5) suggesting increased inputs from intensification and readily-metabolizable inputs from legumes supports SOM formation essential to sustain productivity through POM turnover and confers long-term persistence as MAOM. This demonstrates the importance of not only increasing productivity to promote greater SOC, but that which crops are included in the rotation could significantly affect the stability and potential to gain SOC. We further tested this idea through the MEMS 1.0 simulation to assess the long-term potential of these rotations to influence SOC and whether our proposed mechanisms were consistent with the MEMS framework for SOM formation.

MEMS 1.0 Model Performance

The MEMS 1.0 simulation captured measured SOC values for 2018 and the relative SOC distribution across SOM fractions well (Fig. 2.6), though poorly predicted values for individual sites or fractions (Appendix Table 2.5). The largest discrepancy between measured and modelled values was in the MAOM and lPOM fractions. The model overestimates of MAOM were driven primarily by the Akron ARS site where SOC values were lower than modelled values for all soil fractions (Appendix Fig. 2.1). This site has been previously found to be unusually unresponsive to changes in crop rotation (Bowles et al., 2019) compared to other long-term rotation studies, perhaps due to a combination of climate and soil characteristics (e.g. low surface pH). The model predictions are not biased by rotation or climate region (Appendix Fig. 2.1).

As our model estimates for POM were based on literature values of plant chemistry and biomass estimates calculated from grower-reported yield data, the remaining measured and modeled discrepancies may be due to the relatively large uncertainty in model inputs rather than the model calculations themselves. Additionally, it may be that the equations for fragmentation and perturbation [e.g., Scheu and Wolters, (1991); Yoo et al., (2011)] of litter used in MEMS 1.0 do not capture lPOM loss from wind or water erosion as a portion of aboveground biomass may blow off-site (Duly, 1958). Measured DOM was consistently nine times higher than the modeled pool, suggesting the model systematically underestimates this dynamic C pool which was not calibrated with measured values in the original testing (Robertson et al., 2019). It could also suggest that the way we measure DOM (shaking soil in water for 15 minutes and centrifuging to remove solids) over-samples the amount of soluble C realistically available in the soil at any given time. While model projections of total soil C show notable variability depending on the site as well as the rotation, this variability is consistent with other model simulations of similar agroecosystems in the same region (Robertson et al., 2018). This other study did not explicitly discuss the impacts of leguminous crop rotations and used the DayCent model instead of MEMS to simulate the top 20 cm, but similar trends in bulk soil carbon stocks were seen for the FG-L and continuously cropped rotations (which included CG-L and CG+L).

Future soil C scenarios

To estimate the long-term effect legumes may have on SOC in the region, we used the MEMS 1.0 model to simulate changes to SOC fractions upon conversion from FG-L to

CG+L and CG-L rotations. Despite being a simple model of SOC dynamics, not including, for example, water or N cycling (Zhang et al., 2021), a valuable feature of the MEMS 1.0 is that the C pools simulated by the model are measurable and thus comparable directly to field observations. For the sites in this study, the MEMS 1.0 simulation had lower-than-expected SOC losses during the spin-up period compared to those observed in field studies examining historic FG-L rotations (Peterson et al., 2020). Our C input values for the spin-up also reflect FG-L under no-till conditions which only became possible in the 1960s and widespread in the Northern Great Plains in the early 2000s (Claassen et al., 2018), thus the model likely receives higher C inputs and retains more residue that was historically true for the fields in this study (Sirisha et al., 2019). The main purpose of the spin-up was to establish steady-state conditions comparable among sites to assess the effect of a continuous cropping system rather than to capture the exact conditions likely experienced by fields over the simulation time-period.

The simulated conversion from FG-L to continuous rotations demonstrated the potential of these rotations to increase SOC. The modeled SOC values responded quickly to the increased inputs of the CG-L and CG+L rotations primarily as increased C inputs to lPOM and hPOM (Fig. 2.7). The fluctuations in total C stabilized within 24 years (6 rotation cycles), and any further, long-term changes to total C were due to changes to the MAOM C pool. By 2100, CG+L recovered to the initial starting SOC values and contained 43% more total C than FG-L with 60% of that difference due to MAOM. The CG-L, in contrast, had 33% more SOC than FG-L in 2100, though only 30% of that increase was due to MAOM. The simulations from MEMS 1.0 corresponded well to our measured C pools after two rotation cycles for all but one site, suggesting that the mechanism explored through this study and as the premise for the MEMS 1.0 model for SOM dynamics has merit. The implication is that providing more, continuous, and diversified soil C inputs can stimulate POM turnover supporting N availability for increased yields, at the same time as long-term increases in SOC primarily as MAOM.

The simulation also points to the limitation of management to build or restore SOC through intensifying or diversifying annual crop rotations. It took nearly a century of legume cultivation for the simulated CG+L rotation to recover its modest initial starting SOC value, and the CG-L rotation reached steady-state conditions below the initial values. The CG-L fields also showed the greatest variability in response, with some sites continuing to lose C after conversion. These tended to be the sites with lower average yields and less precipitation, underlying the importance of having sufficient C inputs to sustain SOC levels. The conventional practice of FG-L lost soil C at every site, suggesting that the input amount and frequency was not sufficient to prevent steady SOC losses (rate of 10 kg C ha^{-1} yr^{-1}). Other studies have found that continuous loss of SOC with cultivation (e.g. Senthilkumar et al., 2009), but that diverse rotations (Ladha et al., 2011), N fertilizer (King and Hofmockel, 2017; Salter and Green, 1933), and inclusion of legumes (Heenan et al., 2004; Poffenbarger et al., 2020) slow the loss of SOC by 3-50%.

The rate of SOC loss and effect of management crucially depends on the relative proximity to steady-state conditions a soil system is in when change in management takes place (Soussana et al., 2006). Management effects induce a greater magnitude of response in SOC if implemented when SOC is at equilibrium, but regardless of the underlying direction of change in SOC, the amount of SOC gained from improved management practice may be the same (Sanderman and Baldock, 2010). Thus, based on our measured and modeled scenario, adopting a continuous rotation with or without legumes could provide ~16% gain in surface SOC within a few decades if C inputs are >3 Mg C ha^{-1} yr^{-1}.

5. Conclusion

Our study demonstrated that incorporating legumes into continuous grain rotations in the US central Plains can address the 'soil C dilemma' by coupling increased C input from intensification with increased legume N inputs to support greater SOC and sustain productivity. Our integrated observational-modeling approach demonstrates that the form of increased inputs to soil are relevant to the formation and persistence of organic matter in this semi-arid agroecosystem. Coupling increased C input with N in a readily-metabolizable form facilitated greater stocks of both MAOM and POM, whereas increasing primarily C even with N-fertilizer did not result in similarly larger SOC stocks. As our findings were limited to the soil surface, to see deeper gains of SOC may require incorporating plants that have a high proportion of metabolic compounds and deep root systems. Some farmers in eastern Colorado are experimenting with this strategy by incorporating leguminous, perennial cover crops as Dutch white clover (*Trifolium repens*) as a living cover between small grain harvests, a practice made possible with modern seeding and no-till technology. Our study reveals, however, that incorporating peas in cereal rotations may offer greater potential for SOC gain than feasible through increasing N through fertilizer alone. Future research should focus on mechanisms of how legume cultivation and residue can promote enhanced SOC formation as well as the potential for incorporating perennial plants as cover crops or for forage in integrated livestock systems to rebuild SOC at depth. Additionally, the wide variability among sites suggests a need to better understand the interacting effects historical management, soil mineralogy, and climate may have in influencing response of SOC to management. Given the urgent need to adapt to less favorable growing conditions as climate change intensifies and to develop regenerative agricultural systems, researchers need to partner with growers in identifying the most effective management for sustaining food production and ecosystem function in semi-arid regions.

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CHAPTER 3: PERENNIAL GRAIN KERNZA® FIELDS HAVE HIGHER PARTICULATE ORGANIC CARBON AT DEPTH THAN ANNUAL GRAIN FIELDS [3]

1. Introduction

Annual grain agriculture accrues a soil organic carbon (SOC) debt due to the paucity of inputs and reliance on frequent disturbance compared to the displaced natural ecosystems whose perennial vegetation built stores of SOC. Converting from annual to perennial grain crops offers the potential to repay that SOC debt as such systems have minimal disturbance, reduced erosion, deeper root systems, and longer periods of plant growth (Crews et al., 2018). Studies measuring the rate of SOC accumulation when converting from annual cropping to grassland or perennial biofuel vegetation range from 0.3 to 1.88 Mg C ha^{-1} yr^{-1} (Crews & Rumsey, 2017), leading perennials to be promoted as a SOC sequestration strategy. Perennial vegetation may increase SOC in two main ways: (1) by providing continuous, living cover to the soil, it can reduce erosion and enhance SOC inputs, and (2) with deeper and more robust rooting systems, perennial vegetation adds SOC to depths where it is more likely to become stabilized root debris, may undergo slower decomposition due to reduced microbial activity in subsoils, and root exudates may bond to the more available mineral surfaces. Fresh root inputs at depth, however, may also prime the mineralization of existing SOC and result in little or no net SOC increase.

Kernza®, a grain produced by domesticated forms of intermediate wheatgrass (*Thynopyrum intermedium*), is grown on 1600 hectares in the U.S. and is the only perennial grain available to U.S. farmers currently. While the sequestration potential of Kernza production has

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been estimated using net ecosystem exchange flux measurements[4], no studies have yet quantified the effect of conversion of annual grains to Kernza on SOC stocks to depth at a field scale.

We conducted an observational study to assess whether Kernza fields increased SOC compared to annual row-crops by sampling three fields with the longest known history of Kernza cultivation in Kansas, U.S. and adjacent fields remaining under annual grains. Detecting changes to SOC is difficult due to the inherent heterogeneity of soils and the long time-horizons for change. Examining SOC stocks as distributed between chemically and functionally distinct fractions of mineral-associated [MAOM (< 53 μm)] and particulate organic matter [POM (> 53 μm)] enhances our ability to detect differences, especially for the relatively small POM fraction (Cotrufo & Lavallee, 2022).

POM originates from fragmented plant and microbial structural debris (de Oliviera et al., 2018). Unless it is occluded in stable aggregates, chemical recalcitrance is its only form of protection from further microbial decomposition in agricultural soils, making it vulnerable to disturbance such as tillage. As POM generated from grass residues has a C:N higher than that of the microorganisms feeding on it, POM decomposition likely has a low microbial use efficiency and results in little long-term SOC accumulation. However, POM formed from root input in subsoils can accumulate due to the limited microbial activity and stabilize by aggregate occlusion.

In contrast, MAOM forms from root exudates and residues' leachates either through direct sorption to minerals or after microbial utilization and subsequent adsorption of microbial necromass to minerals. Due to its origins, MAOM tends to increase with microbial turnover and plant inputs with high proportions of metabolic components and N such as from legumes

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(Cotrufo and Lavallee, 2022). The mineral-association renders much of the MAOM-C inaccessible to further microbial processing and resistant to disturbance. Therefore MAOM-C is on average older than POM-C, and it accounts for the majority of SOC in annually cropped agricultural ecosystems (Cotrufo & Lavallee, 2022 *and references therein*).

In order to better appreciate the effect of conversion to Kernza on SOC and N dynamics, we quantified the amount of SOC and N as MAOM and POM in four depth increments from 0-100 cm. We hypothesized that perennial vegetation would have greater overall SOM at depth as well as a higher proportion of C as POM due to the deeper rooting systems of perennials and the tendency for root tissues to contribute chiefly to POM. Given our perennial fields were not intercropped with legumes, we did not expect significant MAOM increases.

2. Methods

Experimental Site and Design

The three study sites were chosen for having adjacent plantings of Kernza and annual crops. Two sites were farms, while the third was an experimental research trial including a restored native prairie treatment. Details on study sites are provided in Table 3.1. All sites are located in Kansas, US on silt loam soils and a typical continental climate.

Soil Sampling

Annual and Kernza fields were sampled by NRCS soil survey crews in December 2019 using stratified random sampling based on soil type from the USDA Web Soil Survey using a truck-mounted, 3.81 cm diameter hydraulic probe (Giddings Machine Company, Inc Windsor, CO). Three samples from each soil type were collected at the vertex of an equilateral triangle with side lengths 6 m, and each sample consisted of three, composited cores sampled at the vertex of a equilateral triangle with lengths of 1 m (Spencer et al., 2011). Each core was divided into four depth increments (0-15, 15-30, 30-60, 60-100 cm) and composited in the field.

Laboratory Analyses

Soils were ground by hand using a weighted wheel to a maximum size of 2 mm and dried at 35-37°C within one week of collection. Moisture content was determined on a sub-sample of ~20 g soil dried at 120°C. We calculated bulk density based on the total sample mass minus soil water content in the core volume calculated from the sample depth increment and soil core diameter. Coarse fragments >2 mm were negligible.

Further, we separated POM and MAOM by wet-sieving at 53 µm ~8 g of oven-dried soil after mechanical dispersion by shaking with glass beads and 30 mL of 0.5% sodium hexametaphosphate for 18 hours (Cotrufo et al., 2019). Mass recovery after fractionation ranged between 99 and 102%.

As many samples contained inorganic C (IC), samples that produced bubbles (CO_2) with two drops of 1M hydrochloric acid were acidified prior to analyses on the elemental analyzer to remove all IC. We quantified the %C and %N of bulk soil, POM, and MAOM fractions on an elemental analyzer (CN analyzer Costech 4100, Italy). Fraction C and N recovery compared to bulk soil were: C mean 101%; N mean 98%. Mid-infrared (MIR) spectroscopy was used to estimate soil pH and percent clay (Seybold et al., 2019) which were used in the regression analysis.

Statistical Analyses

We assessed the effect of crop on SOC and N using mixed-linear effects model of Analysis of Variance (ANOVA) with Kenward-Roger correction. We log-transformed the SOC and N data to account for the non-normal distribution of residual values and analyzed each fraction separately. Fixed terms in the model included the categorical effect of crop, depth, interaction of crop and depth, and the continuous variable of MIR-estimated percent clay. We created a random variable that combined the site with the soil-stratification pairing to account for variability introduced by site and soil history.

As the regression model cannot account for non-linearity in soil profiles at depth and the non-independence of vertically nested measurements, we additionally tested for differences in SOC using bootstrapped resampling with local least-squares-based polynomial smoothing (LOESS) regression (Keith et al., 2016). We calculated the cumulative amount of SOC for each sample using an equivalent soil mass (ESM) approach (von Haden et al., 2020). Using these values, we created a bootstrapped population based on combined samples from under Kernza and annual crops on SOC with replacement (n=1000). We used the bootstrapped dataset to generate a 95% confidence interval (CI) for the combined management and used a LOESS regression to model the effect of the Kernza cropping system on SOC.

3. Results and Discussion

We set out to assess the effect of Kernza on SOC stocks, hypothesizing that the perennial vegetation would lead to enhanced SOC primarily as POM due to its deep root system and year-round presence. We found bulk SOC was higher under Kernza than in annual cropping systems (*t=2.59, p=0.03*) with fields planted to Kernza having ~ 4±2 Mg ha^{-1} more SOC than annually cropped fields across the 0-100 cm soil profile. There were no differences in bulk soil N. Assuming fields planted to Kernza had similar soil C to the annual fields at planting, this would suggest an average SOC gain with Kernza of 0.4 ±0.2 Mg C ha-1 yr-1 which is aligned with other estimates of converting to perennial vegetation (Crews & Rumsey 2017). The SOC accrual rate under Kernza may have been faster as farmers described selecting Kernza fields in part due to low yield expectations for annual crops in those areas, suggesting the assumption of similar starting SOC values may not be valid.

Examining the MAOM and POM fractions provided insight into how the annual and perennial vegetation impacted SOC and N in soil (Fig. 3.1). Both POM-C and POM-N were higher in Kernza fields ($t=19.26$, $p<0.001$ *C*; $t=4.51$, $p<0.001$ *N*) compared to those annually cropped (Table 3.1). The same was true for POM-C in the restored native prairie ($t=2.75$, $p=0.02$), though not POM-N ($t=1.57$, $p=0.3$). MAOM-C tended to be higher under Kernza ($F=2.6$, $p=0.07$), though MAOM-N did not vary by vegetation. Given the potential for non-linear changes in soil characteristics and the interdependence of the soil depths, we used the BLR to assess the overall difference in bulk SOC and fractions (Fig. 3.1). We found POM-C was consistently higher for Kernza while there were no differences in bulk or MAOM-C. These findings support our hypothesis that perennial vegetation promotes greater SOC primarily as POM. Soil under Kernza is not tilled after planting, promoting aggregation and reducing microbial access to surface residues. Reduced mixing and aggregate occlusion could promote greater POM in the surface soils while the deeper root structural inputs could enhance POM formation at depth. There was a greater difference in POM-C in the two sites with regular tillage, supporting the hypothesis that reduced tillage promotes the preservation of POM-C (Cotrufo & Lavallee, 2022).

As root exudates and their associated microbial communities are known to support MAOM formation, we may have expected Kernza also to enhance MAOM-C with its deeper, denser, and longer-lived root systems (Cotrufo and Lavallee, 2022). We saw little change in

MAOM-C, however. While detecting differences in this larger pool of soil C is more difficult, there may not be significant increases to MAOM despite increased root activity since the additional C without additional N inputs may lead to priming.

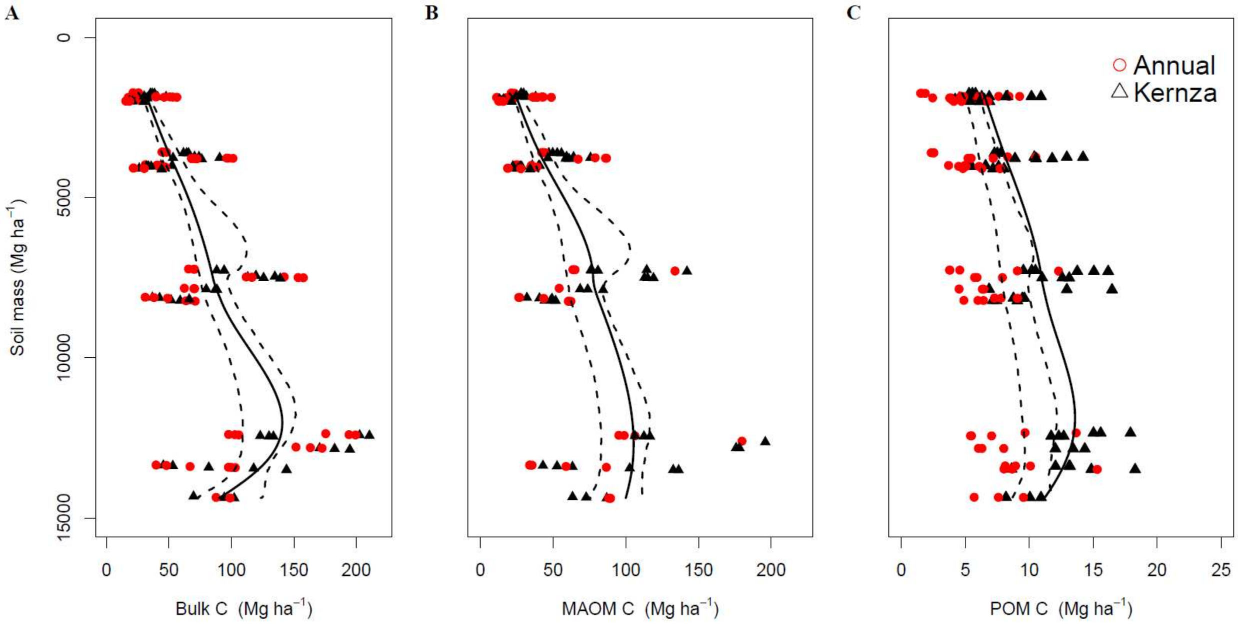

Figure 3.1 Bootstrapped local least-squares based polynomial smoothing (Loess) regression of measured soil organic carbon (SOC) in (A) bulk soil, (B) mineral associated organic matter (MAOM), and (C) particulate organic matter (POM) along the 0-100 cm soil profile. The equivalent soil mass (ESM) portrays to sum of mass over the depth sampled (y-axis) based on the bulk density and estimated mineral mass of soil for each depth increment sampled. The cumulative soil carbon (x-axis) is calculated based on the ESM and percent carbon for each depth and soil fraction. Bulk soil is the whole soil sample; MAOM = mineral-associated organic matter (<53 µm); POM = particulate organic matter (> 53 µm). Triangles represent Kernza vegetation; circles are annual vegetation. Dashed lines represent the 95% confidence interval (CI) based on the bootstrapped sampling (n=1000) with replacement of pooled soil C values from both annual and Kernza vegetation. The solid black line represents the model of soil C from the Kernza vegetation only. Where the model falls outside of the CI suggest significant differences in the two vegetation types.

Table 3.1: Mean soil organic matter C and N stocks by depth. The three sites in this study were managed as annual (A.) crops prior to the establishment of perennial (P.) Kernza in the year indicated for each site. Samples were paired by soil series for the annual and Kernza fields. For C and N stocks, all values are given in Mg ha^{-1};values in parentheses indicate the standard deviation averaged across site for field characteristics and depth for stocks. Est means ‘estimated’ from mid-infrared spectroscopy. POM is particulate organic matter; MAOM is mineral-associated organic matter. Asterisks indicate significant differences based on analysis of variance (ANOVA) between annual and perennial management where *** indicates $p<0.001$ and * indicates $p < 0.05$. $^{\phi}$ This amount is an estimate based on typical application rates in the region. ǂThe Salina site had a buried A horizon ~80 cm depth under the restored prairie.

Field Characteristics			
	Mentor	**Ellsworth**	**Salina**
Soil Classification	Cumulic Haplustoll; Fluventic Haplustolls	Udic Arguistolls; Cumulic Haplustolls	Fluventic Haplustoll
Field Size (Ha)			
Annual	24	10	0.14
Perennial	1	11	0.14
Mean BD (g cm^{-3})			
Annual	1.27 (0.09)	1.42 (0.1)	1.23 (0.1)
Perennial	1.29 (0.07)	1.37 (0.1)	1.23 (0.05)
Mean Est. %Clay			
Annual	42 (19)	24 (9)	31 (10)
Perennial	37 (14)	25 (7)	36 (10)
Mean Est. pH			
Annual	7.77 (0.4)	6.51 (0.8)	7.89 (0.3)
Perennial	7.58 (0.8)	6.77 (0.5)	7.67 (0.4)
A. Rotation	Sorghum-Soy-Wheat	Fallow-Sorghum-Oat-Wheat	Wheat-Sorghum-Soy
A. Management	Annual tillage; winter grazed	No-till; winter grazed	Annual tillage
A. Fertilization (kg $ha^{-1} yr^{-1}$)	80 N^{ϕ}	90 N	84 - 123 N; 56 P
P. Fertilization (kg $ha^{-1} yr^{-1}$)	None	Manure *rate unknown*	None
Year P. Planted	2011	2014	2002
P. Field Prior Use	Annual Cropping	Annual Cropping	Alfalfa

Soil Organic Matter (Mg ha^{-1})							
Depth (cm)	***n* *cores***	**Bulk OC***	**Bulk N**	**MAOM C**	**MAOM N**	**POM C*** **	**POM N**
Annual							
0-15	18	28.7 (11.5)	3.0 (0.9)	23.5 (10.5)	2.5 (0.9)	4.6 (2.1)	0.3 (0.2)
15-30	18	24.3 (12.6)	2.5 (1.1)	21.6 (11.3)	2.3 (1.0)	1.0 (0.3)	0.07 (0.03)
30-60	18	33.1 (16.8)	3.7 (2.0)	26.7 (15.7)	3.5 (1.8)	**1.2***** (0.6)	0.09 (0.05)
60-100	18	32.9 (12.8)	3.9 (1.7)	32.7 (13.8)	3.7 (1.4)	2.2 (3.7)	**0.09*** (0.07)
Perennial Kernza®							
0-15	18	31.1 (8.4)	3.1 (0.6)	23.8 (6.9)	2.6 (0.6)	6.4 (1.9)	0.4 (0.1)
15-30	18	22.6 (10)	2.2 (0.8)	24.5 (9.1)	2.0 (0.7)	1.9 (1.0)	0.1 (0.08)
30-60	18	35.8 (17.8)	3.7 (1.6)	35.0 (19.1)	3.5 (1.5)	**2.8***** (2.3)	0.1 (0.05)
60-100	18	40.9 (20.7)	4.0 (1.8)	39.2 (21.2)	4.0 (1.7)	2.0 (1.0)	**0.1*** (0.1)
Restored Prairie							
0-15	3	33.6 (0.5)	3.40 (0.1)	25.7 (0.9)	2.7 (0.1)	5.1 (0.8)	0.4 (0.04)
15-30	3	24.2 (1.3)	2.55 (0.1)	20.8 (1.8)	2.3 (0.2)	2.0 (0.5)	0.1 (0.03)
30-60	3	24.0 (2.6)	2.63 (0.2)	23.7 (3.8)	2.6 (0.2)	2.4 (0.8)	0.2 (0.02)
60-100	3	52.7 (2.8)†	5.31 (0.3)	51.1 (1.9)	5.3 (0.2)	1.4 (0.5)	0.07 (0.02)

4. Conclusion:

Kernza demonstrated the potential to increase soil C and N at depth primarily as POM on working farms as hypothesized. As POM decomposes readily when conditions are favorable and there were scant differences in MAOM, it is unclear from this study whether planting Kernza monoculture will result in long-term C sequestration. Promoting SOM accrual as MAOM could be enhanced by supplying the perennial system with greater N and labile C inputs such as by inter-cropping legumes with similarly deep root systems as Kernza. As this was a small, observational study initiated after Kernza was established, future studies would benefit from sampling prior to conversion as well as utilizing an approach that combines mechanistic, manipulative experiments and robust field sampling with cost-effective techniques such as MIR spectroscopy. Such work could elucidate the potential for the deep soil POM-C to become occluded in aggregates and be considered long-term SOC storage as well as assess the rate of SOC accrual with this emerging perennial grain. Finally, incorporating the representation of perennial crops in new generation models which represent POM and MAOM dynamics is auspicable to forecast their effects of SOC stocks and soil health and inform their adoption at scale.

Data availability:

Data are available in the GitHub repository, [DOI: 10.5281/zenodo.6588654, https://github.com/slylyvp/KernzaC].

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CHAPTER 4: QUANTIFYING SOIL ORGANIC MATTER FORMATION AND STABILIZATION FROM ABOVE- AND BELOW-GROUND RESIDUES UNDER CONTRASTING NITROGEN MANAGEMENT IN A PERENNIAL GRAIN AGROECOSYSTEM[5]

1. Introduction

Regeneration of soil organic matter (SOM) in arable land is considered as one of the best natural based solutions to mitigate climate change (Paustian et al., 2019; Bossio et al., 2020) and sustain food production (Schipanski et al., 2016). Perennial grains hold many promises: they have the potential to reduce erosion and the need for weed management (Crews et al., 2018), have high water-use-efficiency and known tendency to act as a carbon (C) sink (de Oliveira et al., 2020), and have been shown to have increased resilience and fewer synthetic inputs when intercropped with a legume (Crews et al., 2022). For these reasons, not only are perennial grain systems likely to require fewer inputs, but they may also promote soil organic C (SOC) sequestration through their deep, robust root systems. Yet, we have limited knowledge on how to optimize SOM formation and persistence in perennial grain systems. We have demonstrated the importance of perennial roots in the formation of particulate organic matter (POM) (van der Pol et al., 2022a), the fraction of SOM that is chiefly composed of structural, fragmented and partially-decomposed plant materials (Lavallee et al., 2020). Since the POM fraction is more vulnerable to disturbance and decomposes on shorter timescales than mineral-associated organic matter (MAOM) (Cambardella and Elliott, 1992; Poeplau et al., 2018), an unanswered question is how to promote the regeneration of both POM and MOAM. Restoring both portions of SOM has benefits to soil health in an agricultural context as the continuous turnover and replenishment of POM stimulates nutrient cycling and microbial activity important for sustaining production,

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[5] Publication pending.

while the slower cycling of the MAOM fraction can lead to long-term SOC sequestration (Janzen, 2006; van der Pol et al., 2022b).

In a perennial agricultural context, a fundamental management decision to achieve both increased SOC and grain production is controlling the inputs to the soil through crop(s) and nutrients selection. The crop physiology, life history, and phenology will determine the chemistry, depth, and physical structure of the inputs to soil, especially in a perennial system where tillage is restricted to windows of re-seeding the crop every four or five years. The role plants and their litter chemistry have in SOM formation varies by ecosystem (Khan et al., 2016), is sometimes not observed at all (Gentile et al., 2011), and is intertwined with the soil microbial community (Van der Heijden et al., 2008; Grigulis et al., 2013). The inconsistency hampersour ability to form an overarching framework for how to predict and consider how plants influence SOM (Hall et al., 2020b). Nonetheless, litter chemistry has proven important to the rate of decomposition and formation of SOM in many contexts (Hobbie, 2015; Mueller et al., 2015; Almeida et al., 2018; von Haden et al., 2019; Huys et al., 2022). Litter chemistry influences SOM dynamics largely through the proportion of soluble compounds, of N and of the acid unhydrolyzable fraction (AUR) (Talbot and Treseder, 2012) which is often used to describe complex, aromatic compounds such as lignin, cutins, tannin, and suberin that require specialized oxidation enzymes to decompose (Hammel et al., 2002; Rasse et al., 2005). Litter tissues with a high proportion of structural compounds have long been thought to limit litter mass loss (Berg and Staaf, 1980; Melillo et al., 1982), lead to greater CO_2 losses during decomposition due to low microbial-use-efficiency (as low as 1% (Stott et al., 1983) - 8% (Bahri et al., 2008)), and contribute little to mineral-associated organic matter (MAOM) (Kögel-Knabner et al., 2008; Grandy and Neff, 2008). This litter with higher AUR/N would primarily contribute to particulate organic matter (POM) over time (Soong and Cotrufo, 2015; Cotrufo et al., 2015). In contrast, litter with substrates easily metabolized by soil microbes, that is compounds which are soluble and/or high in N, might be most efficiently stabilized in soil as MAOM either through direct association (Islam et al., 2023), or because a greater fraction of that residue might be converted to microbial biomass than is lost as CO_2 (Cotrufo et al., 2013; Bradford et al., 2013). Through this framework, litter with a low AUR/N and higher proportion of soluble components would preferentially form MAOM (von Haden et al., 2019). In this way, introducing management practices that increase the litter inputs with low AUR/N could promote SOC accrual and stabilization as that could enhance the microbial community carbon-use-efficiency (CUE; Kallenbach et al., 2019) as well as microbial-derived-C which is preferentially stabilized (Kallenbach et al., 2016).

An alternative hypothesis is that low AUR/N litter may lead to little or no SOC accrual by increasing the rate and proportion of litter that is decomposed (Vesterdal et al., 2008; Mueller et al., 2015). This possibility is still consistent with enhanced microbial-efficiency of low AUR/N litter as even though a greater proportion of the high quality litter might be stabilized, the faster and more complete decomposition also may result in greater respiration and the same (Córdova et al., 2018) or lower SOC stocks (Vesterdal et al., 2008). This might occur in microbial communities with higher CUE if the higher CUE is combined with faster microbial turnover, and the more efficient internal recycling of necromass results in less retention of litter-derived C (Córdova et al., 2018). These hypotheses are based on the dominance of the *in vivo* pathway of SOM formation, which is where microbe-synthesized compounds dominate MAOM formation (Liang et al., 2017). While this pathway may dominate in most soils (Miltner et al., 2012; Gillespie et al., 2014; Córdova et al., 2018; Angst et al., 2022), plant-derived compounds could also be directly stabilized through the *ex vivo* modification pathway (Liang et al., 2017). In this pathway, extracellular enzymes depolymerize plant compounds which are not readily palatable to microbes and thus can form MAOM (Angst et al., 2017). This pathway may explain evidence that lignin may decompose faster than total SOC in early stages of decay (Thevenot et al., 2010) while limiting later-stages of decay (Hall et al., 2020a). Lignin may also be enriched in MAOM depending on the redox and iron phases present in the soil matrix (Wan et al., 2019; Huang et al., 2019), potentially through this *ex vivo* mechanism. In this way, litter quality may influence the relative importance of the *in vivo* and *ex vivo* pathways of SOM formation which could be interpreted based on the C:N of the SOM fractions with higher C:N suggesting plant-origin of SOM (Liang et al., 2017; Córdova et al., 2018).

While forming SOM is key to regenerating cropping systems, achieving sustainable and resilient food systems requires reducing reliance on non-renewable and environmentally harmful inputs and supporting enhanced ecological services such as efficient nutrient cycling and biodiversity (Crews, 2013; Altieri et al., 2015; Schipanski et al., 2016). Enhancing diversity of the agroecosystem is a fundamental principle that has been demonstrated to improve ecosystem services in many dimensions (Balvanera et al., 2006; Hooper et al., 2012; Lange et al., 2015; Renard and Tilman, 2021), and for perennial systems diversification is practically achieved through intercropping (Jensen et al., 2005; Drinkwater et al., 2021). Intercropping perennial grains with legumes offers a promising approach to achieve a resilient, low-input with efficient N recycling, and productive agroecosystem (Crews, 2005; Crews et al., 2022), though there are few studies of the ability of perennial grain and perennial grain intercrops to enhance SOM.

Theoretically, intercropping legumes into perennial grain systems could enhance SOM formation by supplying soil with both high and low AUR/N residues to the soil both above and belowground. This could promote both POM and MAOM formation, according to the two-pathway model of SOM formation (Cotrufo et al., 2015), as may have been observed in some annual grain systems through crop rotation (van der Pol et al., 2022b). The effects of legumes on SOC are variable, however. Many studies have found evidence that legumes with their low C:N tissues not only facilitate subsequent or intercropped grains (Fornara and Tilman, 2008; Hauggaard-Nielsen et al., 2008; Nyfeler et al., 2011; Duchene et al., 2017; Crews et al., 2022), but that they can also promote SOC accrual (Drinkwater et al., 1998, 2021; Puget and Drinkwater, 2001; Bichel et al., 2016). Other studies, however, have found legumes had no effect on SOC (Carranca et al., 2009a; Gentile et al., 2011; Chen et al., 2018) or may even reduce SOC due to reduced soil inputs as legume crops have lower biomass than commodity grains they might replace in rotation (King and Blesh, 2018). The inconsistent effect of legumes on SOC highlights the uncertainty and variability in how litter chemistry and decomposition interact within a given soil and ecosystem to influence SOM.

We set out to test the effect of litter inputs on SOM formation under contrasting N management of the intermediate wheatgrass (IWG) *Thynopyrum intermedium* which is being domesticated for perennial grain production as Kernza®. Using continuously labeled $^{13}C/^{15}N$ plant residues of the root and shoot material of Kernza and perennial legume alfalfa (*Medicago sativa*), we studied how litter inputs of contrasting composition form SOM in stands of Kernza where N was (1) not added, (2) added as urea (100 kg N ha^{-1} yr^{-1}), or (3) fixed by an alfalfa intercrop. We traced the formation of SOM from the labeled litter into soil fractions of free POM (POM), that is POM not protected within soil aggregates, occluded POM (oPOM) which is POM within aggregates, and MAOM (Haddix et al., 2020). We also used the formation efficiency of SOM from plant residues to estimate the soil C formation budget for these systems based on the average productivity from 2016 through 2020 as well as estimates of root biomass below 0-10 cm. Our central questions are:

- How do perennial grains form SOM?
- To what extent is the SOM formation from root vs. shoot material?
- How is SOM formation influenced by N management?
- How does the quality of the litter influence SOM formation?

Under these management practices, we contrasted Kernza monoculture (fertilized and unfertilized) with a Kernza-alfalfa intercrop where the alfalfa was mowed one to three times each season and the aboveground material left as a residue on the surface as a green manure.

We hypothesized that the higher quality (low AUR/N) litter from alfalfa shoots would lead to greater MAOM formation due to its higher density of metabolic components promoting enhanced microbial efficiency and the stabilization of soluble compounds to mineral surfaces. We hypothesized the litters with higher AUR/N (e.g., root tissues and Kernza more than alfalfa) would contribute more C to POM and would have slower decomposition rates, increasing the likelihood of these materials becoming incorporated into soil aggregates oPOM. Overall, we anticipated the majority of SOM formation to occur with root tissues as has been previously documented especially in field settings (Rasse et al., 2005; Poirier et al., 2018) due to a combination of greater contact with the soil surface and decreased palatability to decomposers.

We predicted that the management with N addition may enhance MAOM-formation by alleviating microbial N-limitation and leading to enhanced microbial C use efficiency. Previous studies have found that fertilizer-N may slow decomposition and lead to higher SOC (Riggs et al., 2015) while legume-incorporation provides low AUR/N tissues for efficient microbial metabolism (Cotrufo et al., 2013). Results of this study help clarifying the mechanisms of SOM formation in a perennial grain system, quantify the potential of the emerging perennial grain Kernza to promote soil C accrual, and evaluate the potential for legume intercrop to replace synthetic N inputs while sustaining productivity and SOM.

2. Methods

Field site description and management

This incubation took place in experimental research plots established in 2015 and maintained by The Land Institute near Salina, Kansas, USA (38.770284 N, -97.591795 W); this land was originally home to at least five indigenous nations including Kaw, Osage, Comanche, and Pawnee Nations (https://native-land.ca/). The site is located 370 m above sea level with annual precipitation averaging 737 mm (one tenth as snow). Rainfall is concentrated during the spring and fall with common summer drought. The mean average temperature is 13.2˚C with daily average lows of -6.6˚C in January and average highs of 35˚Cin July. The experimental plots are situated on Cozad and Hord silt loam soils (Soil Survey Staff,).

Prior to use for research, the site was cultivated under annual wheat production until 1990 (Barker and Piper 1994) and a series of cover crops and, Kernza (*Thynopyrum intermedium*), and biennial sorghum for the following 13 years, prior to 2015. In 2015, a randomized block design (RBD) consisting of four blocks with twelve treatments was established to compare legume intercrop varieties, fertilizer management, and crop spacing of Kernza. Each plot in the RBD measured 3.6 x 3.6 m^2 and was planted with a drill depth of 1.2-2.5 cm and a seeding rate of ~11-13 kg ha^{-1}. Plots were weeded by hoe twice in the establishment year and spot-weeded occasionally as needed to remove individual weeds subsequently.

The treatments utilized in this study consisted of: (1) unfertilized Kernza monoculture (KKU), (2) fertilized Kernza monoculture (KKF; 100 kg N ha^{-1} yr^{-1} as urea), and (3) unfertilized Kernza biculture with alfalfa (*Medicago sativa*, StarSeed A100 variety; KAU). The row spacing in each plot was 30 cm with alfalfa rows replacing alternating rows of Kernza in KAU . In the KKF treatment, fertilizer was broadcast by hand each spring between April 15 and May 15. Alfalfa rows in plots were mowed 2-4 times each summer with the residue left on the soil surface after each cutting. There was a gopher infestation that targeted the KAU plots the summer of 2019; by 2020 there was little detectable alfalfa remaining in most plots. Kernza harvest took place in the third week of July using a rice binder.

Aboveground net primary productivity (ANPP) was estimated similar to Crews et al., (2022). Measurements were taken for three years (2016-2018) prior to the start of our experiment and one year during the experiment (2020). Notably, 2018 was a severe drought for the region (National Integrated Drought Information System (U.S.) drought.gov). We calculated ANPP based on the dry weight biomass divided by the row length for each plot based on two harvested rows of Kernza (~2 m^2) and five rows (e.g. all rows) of alfalfa (KAU only) (5.4 m^2) per plot. Biomass was weighed using a field scale with a subsample bagged, dried at 60˚C for 48 h, and re-weighed to determine moisture content.

We estimated N balance for each year with ANPP data as the N inputs as fertilizer or alfalfa N-fixation minus the N exported by ANPP harvest. Aboveground N-fixation (N_{fix}) was estimated based on a linear regression equation (R^2=0.91, n=120 farms in Europe) (Carlsson and Huss-Danell, 2003) for alfalfa based on the dry matter (DM) yield (kg ha^{-1} yr^{-1}):

$$N_{fix}=0.021*DM+17 \qquad \text{(Eq. 1)}$$

To estimate the proportion of N from fixation in alfalfa roots, we used a root factor of 1.61 (Crews et al., 2022) to estimate belowground biomass and estimation that N-fixed in roots is 80% that of shoots (Walley et al., 1996). We also calculated the relative yields (RY) to compare the ANPP of the intercrop and monocrops (Vandermeer, 1990; Crews et al., 2022) as follows:

$$RY = ANPP_{intercrop} / ANPP_{monocrop} \qquad \text{(Eq. 2)}$$

RY values >1 indicate productivity of the intercrop is greater than the monocrop in an equivalent area, implying facilitation, while RY <1 indicates lower productivity in the intercrop compared to monocrop and suggests competition (Vandermeer, 1990).

Background Soil Analyses

At the time of setting up our experiment as described below, we collected samples from each plot for native litter and soil background characterization. Three points within each plot were randomly chosen for litter and soil core sampling. We quantified aboveground litter biomass by collecting and weighing aboveground litter within a 0.04 cm^2 quadrat. We estimated bulk density (0-10 cm) using cores collected with a slide-hammer corer (6.4 cm diameter) by dividing fresh sample mass minus soil water content by the core volume; coarse fragments >2 mm were negligible. Average bulk density in the field was 1.15 g cm^3. On the cores collected for bulk density, we measured soil pH using a 1:5 soil to water ratio and soil texture based on the hydrometer method (Gee and Bauder, 1986). We tested for inorganic carbon presence at the 0-10 cm depth using an acid-fizz test with 1M hydrochloric acid and did not find any.

To quantify and characterize the SOC and soil N stocks for the different management in this study, we conducted physical fractionation of the SOM into two size fractions: POM (>53 µm) and MAOM (<53 µm) after dispersing aggregates by shaking ~8 g of 2 mm-sieved soil samples for 18 h with glass beads in a 0.5% sodium hexametaphosphate solution (Cotrufo et al., 2019). Oven-dried (60˚C, 72 h) fractions and bulk soil were ground to a powder using mortar and pestle and analyzed for total C and N by dry combustion using a LECO Tru-Spec CN gas analyzer (LECO Corp., St. Joseph, MI, USA).

Production of ^{13}C, ^{15}N labeled residues

Kernza (breeding cycle 5) and inoculated alfalfa (Millborn Seed Co) seedlings were grown in a continuous dual isotopic labeling chamber as described by Soong et al. (2014). Plants were grown in 12-L pots with a growing medium of 3:4 ratio of sand and ceramic clay, inoculated with soil from the Kernza-only plots. The air-tight chamber continuously received ^{13}C enriched CO_2 to achieve a concentration of 360-400 ppm and a target label of 4 atom% during the active photosynthetic period. Plants were watered with ^{15}N-labeled Hoagland's solution with increasing fertilizer quantities as plant biomass increased to achieve a target ^{15}N label of 6 atom%. Plants were grown for 3.5 months prior to harvest; alfalfa plants had begun to flower and some Kernza plants had initiated grain production at that time. Upon removal from the labeling chamber, aboveground biomass was clipped at the surface of the growing medium and air-dried. Roots were shaken to remove bulk sand and clay and then rinsed on 2-mm sieves until free of the growing medium and air-dried. A subsample of each plant species and above and below-ground tissue was finely ground on a Wiley mill equipped with a 0.75 mm mesh screen and oven-dried at 40°C for elemental and isotopic analysis as described below. Air-dried plant material was cut to 2 cm and placed and weighed into uniform aliquots for field application at a rate of 494 g m^{-2} for both Kernza and alfalfa roots and shoots (~ 4 g collar^{-1}). This represented at 15% $^{+}/_{-}$ 2% *SD* increase to the soil C per collar by mass. This rate was a compromise between the measured surface litter (Table 4.2) (261 - 593 $^{+}/_{-}$ 84 g m^{-2}), the estimated aboveground net primary production of similar 3-year-old stands of Kernza (470 $^+/_-$ 21 g m^{-2}) and alfalfa (180 $^+/_-$ 19 g m^{-2}), the amount of available labeled root and shoot material for the two crops, and the desire to minimize the proportion of soil C stock the labelled litter represented.

Litter Incubation Experimental design

In order to quantify SOM formation from contrasting plant tissues, we compared the isotopic concentrations of $\delta^{13}C$ and $\delta^{15}N$ in SOM fractions of soil collars with and without added labeled litter to estimate the proportion of litter-derived SOM (Soong and Cotrufo, 2015; Leichty et al., 2020; Haddix et al., 2020). There were four replicated blocks of each management (KKU, KAU, KKF), thus 12 plots overall. Within each plot, we placed fully replicated PVC collars to serve as samples for three measurement points at 3, 12, and 27-months after initial placement. The collars were randomly assigned labeled root or shoot litter or served as a reference control (no added litter) for either roots (‘disturbed’ (DC) or mixed) or shoots (‘undistured' (UC)). The KKU and KKF plots thus each contained 12 collars at the start of the experiment (n=4 per harvest) and KAU plots with the added alfalfa litter, contained 18 collars (n=6 per harvest).

For the incubation, PVC collars (15 cm height, 10 cm diameter) with 0.65 cm diameter holes drilled into the sides to allow root ingrowth were pounded into the ground to a depth of 10 cm. Any plants growing within the collar were clipped at the soil surface and placed within the collar. Root residues (KR, AR) were incorporated with the 0-10cm soil depth by scooping soil within the collar and mixing it within a plastic bag, then returning the soil to the collar and compacting it to return to the original soil volume. Disturbed control collars underwent the same procedure but without receiving litter addition. Shoot residues (KS, AS) were applied to the soil surface. Each collar was covered with a nylon, mesh screen with 2-cm square openings to contain the added litter; these screens were subsequently replaced (Aug 2020) with screens with a 1-cm square openings as the original mesh had the unfortunate consequence of trapping snakes.

Field sampling of soil collars

Soil collars were first installed in May 2019 at a time when the site experienced record rainfall. Collars were collected in August 2019 (3 mo), May 2020 (12 mo), and August 2021 (27 mo). At each harvest, the live plants in the collars remaining in the field for later sampling points were clipped and the aboveground material placed inside the collar. Harvested collars were pulled from the ground intact, wrapped in foil, placed in plastic bags, and stored on ice until refrigerated at 4˚C until processed. The excavated hole was measured at 5 points and averaged to obtain the average sample depth.

Gopher infestation in half of the KAU plots resulted in 3 collars being buried, resulting in deeper soils being deposited on the top. The deposited soil was processed separately, distinguished by its color and reduced density.

Processing and analysis of soil

Each intact sample was weighed to obtain the total mass, minus mass of the collar. Surface litter > 2 mm was carefully removed prior to processing the soil samples, and live plants were clipped at the soil surface. Collars from undisturbed samples (UC, KS, AS) were split in half by depth (0-5, 5-10 cm) to ensure isotopic label could be traced in samples with surface litter application. We did this by carefully scooping soil from the upper half of the sample, weighing each portion, and measuring the remaining soil depth within the collar to obtain the precise volume of each sample portion.

Soils were sieved to 8-mm and a sub-sample for each analyzed for gravimetric water content by mass loss after drying for 48 h at 105˚C. A soil sub-sample was immediately sieved to 2 mm and frozen at -80˚C for microbial analyses. The remainder of the sample was air-dried and then passed through a 2-mm sieve before further analysis. All plant material including live plants, surface litter, and below-ground (root) litter was dried at 40˚C for 48 h, weighed, and ground on a Wiley Mill with mesh size (0.75 mm) for further analyses.

We estimated soil bulk density (BD) of each soil core as described above for the field samples. The soil collar installation resulted in soil compaction that decreased over time as the BD of soil collars increased from the field-level BD by 0.36 g cm^{-3} in undisturbed collars and 0.16 g cm^{-3} in the disturbed treatments at the 3-moth harvest; the differences decreased to 0.12 and 0.07 g cm^{-3} in the undisturbed and disturbed respectively after 27 months. To overcome the changes in BD imposed by the soil collars, we used an equivalent soil mass approach to calculating soil C and N stocks (Ellert et al., 2002). The average soil mass sampled in the collars (0-10 cm) was 1,265 Mg soil ha^{-1} $^{+}/_{-}$ *SD 124*. Soil C and N stocks were calculated by normalizing all samples to the mean sample mass minus one standard deviation (1140 Mg ha^{-1}) to compare equivalent soil mass across soil collars over time.

Litter Chemistry Analysis

To compare the labeled litter material to the Kernza and alfalfa plants growing in the experimental plots, we analyzed litter chemistry for the field and chamber grown plant material. We measured hot water extractable (HWE) C and N, and estimated proportions of acid hydrolysable (celluloses) and acid unhydrolyzable (lignins, tannins, cutins, suberins, etc.) fibers. At the time of the background sampling (May 2019) we collected representative tissue samples from 5 separate plants in each plot (roots to a depth of 10 cm) as well as three lab replicate samples from the labeled litter. For the quantification of HWE, we digested 0.3 g of oven-dried (105˚C) material for 3 h (Tappi, 1981) and measured C and N on a Shimadzu TOC-L/TNM-L Analyzer (Shimadzu Corporation, Kyoto, Japan). For the fiber analyses, we used the acid detergent fiber (ADF) method (Van Soest and Wine 1968). The ADF digestion consisted of boiling 0.3 g of oven-dried (105˚C) material for an hour in detergent solution to remove hemicellulose and non-structural carbohydrates and lipids ('weak acid-soluble'). We then removed acid-soluble fibers (a proxy for cellulose*)* by digesting the remaining material in 73% sulfuric acid. The remaining acid-unhydrolysable residue is considered a proxy for lignin after ash correction (Rowland and Roberts 1994). The labeled litter tended to have a higher proportion of HWE and fewer structural compounds than the plants growing in the field (Table 4.1) as expected with first year plants grown in a greenhouse.

Soil Density & Size Fractionation

We used a density and size fractionation to quantify the formation of newly formed SOM into four chemically and physically distinct pools. The fractionation scheme we followed was as described by Haddix et al. (2020) with the omission of the water-extractable fraction since it was negligible. The measured fractions consisted of three pools: free light POM (fPOM), sand sized and occluded POM (oPOM), and silt and clay-sized, mineral-associated organic matter (MAOM). The sample was gently shaken in a solution of sodium polytungstate (SPT) at a density of 1.85 g cm^{-3}, placed in a vacuum chamber for 10 min to remove air trapped within soil aggregates, and centrifuged at 1069 gravitation for 30 min. We then aspirated off the fPOM and rinsed the remaining soil sample four times with DI water to remove remaining SPT. We separated the oPOM from MAOM by wet-sieving the sample on a 53 μm sieve (oPOM >53 μm, MAOM <53 μm) after dispersing aggregates by shaking the samples for 18 h with glass beads in a 0.5% sodium hexametaphosphate solution. The fractions were finely ground by hand for elemental and isotopic analysis measured on a Costech elemental combustion system coupled to a Thermo Scientific Delta V Advantage Isotope Ratio Mass Spectrometer for %C, %N, $\delta^{13}C$, and $\delta^{15}N$. Mass recovery after fractionation was on average 99% +/- 1.5% SD and ranged from 95 to 103%. After fractionation we achieved an average recovery (n=243) of 89% +/- 11% *SD* for C and of 84% +/- 6% SD for N.

Calculation of SOM formation

We quantified the litter-derived C in each fraction as described by Haddix et al. (2020). We calculated the relative contribution of labeled litter to each fraction (F_L) using the mixing model in Eq. 1 (Balesdent and Mariotti, 1996) and then multiplied F_L by the amount of C in that fraction.

$$F_L = \text{atom}\%^{13}C_m - \text{atom}\%^{13}C_{t0} / \text{atom}\%^{13}C - \text{atom}\%^{13}C_{t0} \qquad \text{(Eq 3)}$$

F_L is the fraction of labeled litter-derived C for each replicate, atom% $^{13}C_m$ is the atom percent ^{13}C in the fraction for each replicate, atom% $^{13}C_{t0}$ is the average ^{13}C in the corresponding control fraction, and atom% $^{13}C_L$ is the average percent ^{13}C of the C in the labeled litter. To reduce the variability in the measurements based on slight differences in C and N concentrations, we averaged the percent C and N for control soil and litter samples by fraction (or litter type) for each treatment. For the control soil samples, we additionally averaged the percent C and N across time to quantify the proportion of litter-derived C to ensure the calculation was not biased by fluctuations in C and N measurement.

Calculation of SOC sequestration rate

In order to scale the litter-derived C estimates to field-level rates of SOC formation in the topsoil (0-10cm), we integrated the productivity and litter-derived data to calculate potential SOC accrual over time (Table 4.5). We based the SOC inputs on the average ANPP values measured 2016-2018 and 2020 by management as well as the C concentration in above and belowground plant tissues measured in May 2019 on field-collected plant samples (Table 4.1). The calculation for the estimated SOC formed from each tissue was as follows:

For shoot tissues:

$$SOC_{S,M} = ANPP_{M} * C_{S,M} * pLDC_{S} * 0.001 \text{ g kg}^{-1} \quad \text{(Eq. 4)}$$

For root tissues:

$$SOC_{R,M} = ANPP_{M} * R{:}S * C_{R,M} * pLDC_{R} * R_{turnover} * pR * 0.001 \text{ g kg}^{-1} \quad \text{(Eq. 5)}$$

where the SOC is the amount of SOC formed from the productivity (Kg C ha^{-1} yr^{-1}) for *S* shoots or *R* roots by *M* management; C is the C concentration in the plant tissue (g C kg^{-1} plant) (Table 4.1), *p*LDC is the proportion of plant litter-derived C recovered as SOC in our labeled litter study, R:S is the root to shoot ratio (0.75), $R_{turnover}$ is the root turnover rate per year (0.5) (Gill and Jackson, 2000), *p*R is the proportion (0.5) of total root biomass in the 0-10 cm soil depth, (Pugliese et al., 2019) , and 0.001 is to convert from g to kg.

Belowground net primary productivity (BNPP) estimates were based off of measurements by Sprunger et al., (2018b) where BNPP was roughly 75% of ANPP and roughly half of the roots were found 0-10 cm (60% from 0-20 cm). Root turnover rate of 50% was based on the estimates in Gill and Jackson (2000) which estimated a 53% annual turnover rate for grassland roots. The SOC estimates by management include C from Kernza roots for KKU and KKF management; for KAU we included SOC from alfalfa shoots and roots as well as Kernza roots. We additionally estimated potential SOC formation in the 0-10 cm depth from the surface litter residues based on the amount of surface litter measured in the plots. Similarly, we estimated SOC formation from Kernza roots from 10-100 cm. The surface litter inputs were based on average litter biomass measured in the field in May 2019 and the average C concentration of that residue across treatment. Root biomass 10-100 cm was estimated based on the average of measured values of root mass in Sprunger et al. (2018b) which ranged from 1005 to 2015 kg ha^{-1} over four years. While rates of SOC formation may be greater at depth than on the surface (Fulton-Smith and Cotrufo, 2019), for the estimate of potential SOC formation from root biomass 10-100 cm we assumed the same formation rates as assessed in this study from 0-10 cm, thus our values are likely an underestimation.

Statistical analyses

We assessed the normality of the data by Shapiro-Wilk test. To determine the extent that management and litter treatment affected the formation and persistence of litter-derived C and N in fPOM, oPOM, and MAOM we ran linear-regression analysis of variance (ANOVA) using R version 4.1.2. We also tested for differences by management and litter type for litter chemistry and ANPP. Negative values (n=17 / 618) for the calculated proportion of litter-derived C and N were converted to zero prior to analyses.

To account for sampling the same plots over time, we included a unique variable that combined the block and management in each analysis. Time, litter type, treatment, and soil fraction were treated as fixed variables, as was the treatment-block variable in cases where the mixed-effects model was singular. For each test of significance of litter type, we included an interaction between litter type, harvest, and, where applicable, soil fraction. We used Tukey-adjusted pairwise comparisons to evaluate the differences in management and litter treatment over time and by litter fraction using the *dplyr* (Wickham et al., 2022), *car* (Fox J., 2019), and *lmer* (Bates et al.,) packages.

3. Results

Litter Chemistry

The litter chemistry of the plants growing in the field under contrasting management were similar in the proportion of structural and metabolic components, %C, and HWE C (Table 4.1). There were higher N concentrations in the fertilized Kernza shoots plants ($P \leq 0.02$, $t \geq 3.1$,) than the unfertilized plots which was also true for root N and HWE N, though only compared to the KKU treatment ($P=0.01$, $t=3.5$). With respect to difference between Kernza and alfalfa and between root and shoot tissues grown in the field, the most notable differences were the higher concentrations of both C and N in alfalfa compared to Kernza, though HWE N was similar for both plants. Kernza and alfalfa species also had similar AUR/N. The LCI differed only for the root tissues where alfalfa had a higher LCI compared to Kernza roots ($P=0.01$, $t=3.4$). Alfalfa and Kernza shoots contained similar proportions of weak-acid soluble components ($P=0.8$), though these more digestible components were ~7% higher in alfalfa roots than those of Kernza ($P=0.02$, $t=3.1$).

The isotopically labeled litter we grew in the growth chamber differed from the plants grown in the field in significant ways, generally having higher C and N concentrations (Table 4.1) and ~12% more weak-acid soluble components than the field-grown plants (P<0.001, $t \geq 5.3$). This translated to the labeled litter also having a lower AUR/N ($P \leq 0.02$, $t \geq 3.0$), though there were no differences in the LCI (range 0.10 to 0.27) between the field and chamber grown plants (P=0.4).

Table 4.1 Summary of litter chemistry for greenhouse-grown labeled litter and plants from each management of the experimental plots in Salina, KS. Plant tissue samples were collected in May 2019. KAU = Kernza-Alfalfa biculture unfertilized management; KKF = Kernza monoculture, fertilized; KKU = Kernza monoculture unfertilized; HWE stands for hot-water extractable; LCI = ligno-cellulose index which is a ratio of the acid unhydrolysable (AUR) to unhydrolysable+hydrolysable (labile) products. N=3 for labeled litter and n=4 for the litter in experimental plots. Letters denote significant differences for $p<0.05$ based on ANOVA.

		Alfalfa		**Kernza**		**Alfalfa**		**Kernza**		**Kernza**		**Kernza**	
		Labeling chamber		**Labeling chamber**		**KAU**				**KKF**		**KKU**	
	Unit	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
At.% C	**‰**	4.2936 (1.1136)	4.4073 (1.1340)	4.388 (1.1385)	4.3436 (1.1764)								
At.% N	**‰**	5.6758 (1.5872)	6.0026 (2.0242)	6.8485 (1.252)	6.6697 (1.4690)								
C	%	**45.00^{a} (0.11)**	**45.24^{a} (1.93)**	**43.88^{a} (0.47)**	**45.25^{a} (1.03)**	43.72^{b} (0.59)	43.03^{b} (1.38)	42.00^{c} (0.73)	42.78^{d} (1.02)	41.97^{c} (0.88)	44.07^{d} (0.52)	41.84^{c}(0.56)	43.40^{d}(0.16)
N	%	**4.54^{g} (0.16)**	**2.91^{h} (0.20)**	**2.18^{i} (0.14)**	**1.82^{j} (0.13)**	2.97^{e} (0.23)	2.29^{f} (0.05)	1.48^{ac} (0.07)	1.02^{bd} (0.11)	**1.9^{a} (0.16)**	**1.02^{b} (0.09)**	1.29^{c} (0.05)	0.79^{d} (0.05)
C:N		8.2^{a}	17.9^{b}	14.5^{c}	30.5^{d}	14.7^{e}	18.8^{f}	28.5^{g}	42.0^{h}	22.1^{i}	43.2^{j}	32.6^{k}	54.9^{l}
HWE C	*mg g^{-1}*	65.31^{a} (7.06)	79.62^{a} (3.36)	62.86^{b} (0.86)	31.56^{c} (1.84)	75.72^{a} (14.26)	56.05^{a} (4.06)	54.15^{b} (4.43)	20.95^{c} (1.54)	57.27^{b} (1.01)	23.8^{c} (1.24)	50.61^{b} (3.21)	24.04^{c} (2.03)
HWE N	*mg g^{-1}*	40.23^{c} (3.66)	61.95^{c} (4.0)	35.24^{a} (2.36)	19.59^{d} (0.5)	60.36^{c} (13.08)	36.02^{cd} (3.9)	46.37^{a} (7.31)	9.12^{d} (0.98)	59.38^{a} (4.01)	10.6^{d} (1.24)	34.79^{b} (2.5)	6.0^{e} (0.64)
Weak acid-soluble	%	**68.32^{a} (0.34)**	**70.15^{a} (0.51)**	**58.25^{c} (0.62)**	**58.13^{c} (0.19)**	52.67^{b} (4.64)	**52.08^{b} (0.39)**	53.14^{d} (1.24)	46.15^{e} (1.36)	55.65^{d} (1.32)	41.88^{e} (0.37)	53.43^{d} (1.4)	42.65^{e} (0.43)
Celluloses	%	25.72^{a} (0.71)	22.73^{a} (0.4)	33.59^{c} (0.66)	26.42^{ac} (2.8)	33.02^{b} (1.89)	31.08^{bd} (1.39)	34.4^{d} (1.14)	33.14^{d} (1.26)	33.07^{d} (1.57)	37.29^{d} (0.96)	32.99^{d} (0.64)	36.36^{d} (1.05)
Lignin-Like AUR	%	5.86^{a} (0.24)	4.9^{a} (0.16)	3.48^{a} (0.06)	9.05^{e} (2.73)	11.08^{d} (1.53)	16.35^{bcd} (2.34)	5.21^{b} (0.51)	13.98^{c} (0.57)	4.54^{b} (0.31)	15.4^{c} (0.85)	5.29^{b} (0.53)	14.1^{c} (0.9)
AUR/N		**1.07**^{a}	**1.87**^{a}	**1.16**^{a}	**6.15**^{c}	3.73^{b}	7.14^{d}	3.53^{b}	13.74^{e}	2.39^{b}	15.12^{e}	4.12^{b}	17.84^{e}
LCI		0.08^{a}	0.07^{ac}	0.06^{b}	0.13^{c}	0.17^{a}	0.24^{ac}	0.09^{b}	0.23^{c}	0.08^{b}	0.27^{c}	0.09^{b}	0.25^{c}

Soil properties and yields across the contrasting Kernza N management

The soils in this experimental site had a large proportion of MAOM (74-91%, median: 84% +/- 0.2 *SE)* and very little fPOM (0.1-1.9%, median: 0.4% +/- 0.02 *SE;* Fig. 4.1). The soil in fertilized management contrasted with the unfertilized in a few ways prior to the start of the incubation experiment. Compared to the unfertilized treatments, soil pH in KKF was lower (Table 4.2; P=0.01 df=6, t=4.2), and it had soil C stocks that were 1.1 and 1.4 Mg ha^{-1} greater than KAU and KKU respectively (Fig. 4.1A). The fertilized plots also had a tendency for higher C:N in the POM and MAOM fractions (Table 4.2). KAU had similar N stocks to KKF (Fig 2.1B), but lower mean C:N in the SOM fractions (Table 4.2).

Surprisingly, we found a small soil texture difference across blocks and treatments as there was a 3% increase in clay and decrease in sand content from block one to four (ANOVA P=0.01 df=3 F=8.6 for sand), such that the plots in blocks 3 and 4 were silty clay loam soils while all others in the treatment were silt loam. The KKU management tended to have ~4% more clay than the other managements (P<0.001 df=6, t>8). The KAU management tended to have ~1.5% $^{+}/_{-}$ 0.6 more sand (P=0.09 df=6, t=2.6) (Table 4.2).

The amount of surface litter varied by management but did not mirror the productivity data (Table 4.2) as the aboveground material for Kernza was removed each harvest and the alfalfa left in the field as a green manure. The biculture plots had the greatest surface litter, followed by KKF then KKU (Table 4.2; P=0.08, df=2 F=3.9).

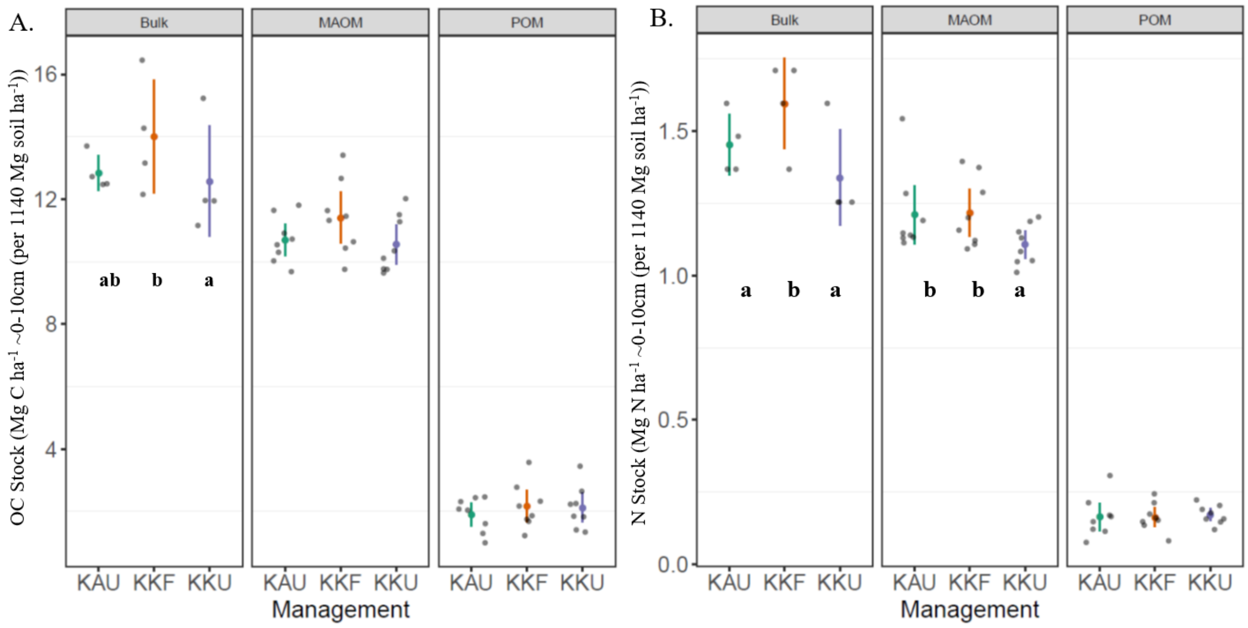

Figure 4.1. Initial (A) carbon and (B) nitrogen stocks for bulk, mineral-associated organic matter (MAOM), and particulate organic matter (POM). These are based on the size-separated fractions prior to the start of the litter incubation in May 2019 and are calculated using the mean soil mass per collar (0-10 cm) which was standardized to ~11 Mg soil ha^{-1}. Letters denote significant differences to $P<0.05$. Abbreviations refer to management: KAU is Kernza intercropped with alfalfa, unfertilized; KKF is Kernza monoculture fertilized at 100 kg N ha^{-1} yr-1; KKU is Kernza monoculture, unfertilized. Central dots represent the mean values by management and the lines represent the standard error. Grey dots are the measured data (n=2 replicates x 4 blocks).

The aboveground net primary production (ANPP) varied significantly from year to year, with a significant decline ($P<0.03$, t> 2.91, $df=32$) in Kernza production each year measured across managements except between 2017 and 2020 ($P>0.13$, t>2.25, $df=32$) (Fig. 4.2). The KKF management had higher yields than the intercrop KAU in 2016 (P=0.03, $t=2.61$, $df=31$) and than both KAU and KKU in 2020 ($P\leq 0.02$, $t=2.81$, $df=32$).

ANPP tended to decline over time for all managements (Fig. 4.2A), though 2020 had similar or higher ANPP than 2017 and 2018 (a severe drought year). The KKF management had higher overall productivity in 2016 compared to the KAU ($P=0.04$, t=2.61, df=32) and compared to both unfertilized treatments in 2020 ($P \leq 0.02$, $t \geq 2.81$, df=32). There were no differences among managements in 2017 (P>0.7), and while KAU had the highest overall ANPP in 2018, these differences were not large enough to be significant (P=0.12, t=2.05, df=32).

Table 4.2: Management differences across soils prior to the incubation experiment. MAOM is mineral-associated organic matter; POM is particulate organic matter; C is soil organic carbon; N is total soil nitrogen; ANPP is above-ground net primary productivity of Kernza. Values in parentheses are standard error. * indicates a p<0.05 from a tukey-method adjusted pairwise comparison after an ANOVA.

Measurement	**Unit**	**Kernza Monoculture Fertilized (KKF)**	**Kernza-Alfalfa Biculture Unfertilized (KAU)**	**Kernza Monoculture Unfertilized (KKU)**
Percent Clay	%	24.8 (0.3)	23.7 (0.3)	**28.6 (0.3)***
Percent Sand	%	17.2 (0.5)	18.9 (0.5)	17.4 (0.5)
pH		**7.3 (0.04)***	7.1 (0.04)	7.1 (0.04)
Bulk Density	g cm^{-3}	1.25 (0.01)	1.25 (0.01)	1.26 (0.01)
MAOM %C	%	**1.26 (0.08)***	1.06 (0.08)	1.05 (0.08)
MAOM %N	%	**0.128 (0.006)***	0.122 (0.006)	0.114 (0.006)
MAOM C:N		9.8	**8.7***	9.2
POM %C	%	**1.32 (0.08)***	1.11 (0.08)	1.11 (0.08)
POM %N	%	**0.100 (0.006)***	0.094 (0.006)	0.086 (0.006)
POM C:N		13.2	11.8	12.9
Fertilizer Application Rate	kg N ha^{-1}	100	0	0
Surface Litter	g m^{-2}	384 (84)	**593 (84)***	261 (84)

Total (root+shoot) estimated fixation-derived N from the alfalfa intercrop (KAU) ranged from 39 (2020) to 129 (2018) kg N ha^{-1} yr^{-1}. Considering the N balance (Fig. 4.2B), we estimated that there were net N losses from each management and year, with losses averaging 39 kg N ha^{-1} yr^{-1} $^{+}/_{-}$ 22 *SE*, except for 2018. The N supplied, in KAU from alfalfa N-fixation and in KKF by fertilizer application, fell short of the Kernza ANPP export by 7-73 kg N ha^{-1} yr^{-1} and 18-122 kg N ha^{-1} yr^{-1} (Fig. 4.2B) for KAU and KKF respectively. In 2018 (the drought year), both KAU and KKF managements had positive estimated N balance of ~100 and ~73 kg N ha^{-1} respectively.

The relative yield (RY) ratios (Crews et al., 2022) for the intercrop were greater than 1 in 2018 compared to the KKF and in both 2017 and 2018 compared to KKU (Table 4.3).

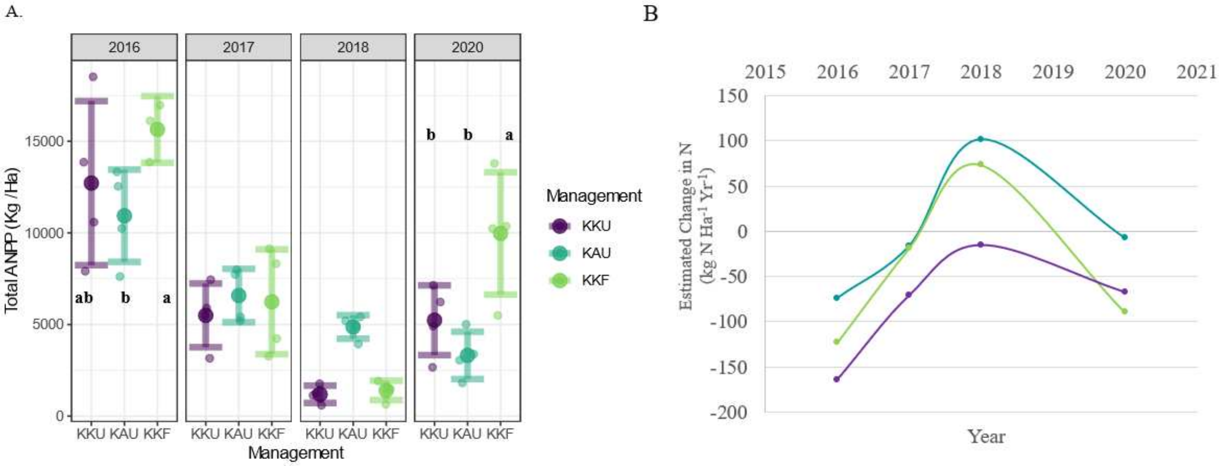

Figure 4.2: A. Annual net primary productivity (ANPP) of Kernza and alfalfa by management for 2016, 2017, 2018, and 2020. No data was collected in 2019. The dots represent the mean dry-weight values of ANPP (grain+aboveground tissues) measured at harvest each year for Kernza and at each mowing event (1-3x growing season) for alfalfa, and the lines represent standard error. Managements are as described in Fig. 4.1. Letters denote significant differences to *P*<0.05. B. Estimated N balance for each cropping systems calculated by subtracting estimates of Kernza N in ANPP from the known inputs of N either from (1) fertilizer application for KKF (100 kg N ha-1), (2) estimated N-fixation by alfalfa in roots and shoots, or (3) nothing in KKU . Aboveground Kernza and alfalfa N was estimated by multiplying the Kernza ANPP (A) by the concentration of N in aboveground tissues (Table 4.1). Numbers below zero mean that we estimated more N was exported than imported based on aboveground inputs while positive numbers suggest net N additions to the soil.

Table 4.3 Relative Yield (RY) ratio of ANPP from the KAU management to the monoculture treatments based on aboveground net primary productivity (ANPP) by year. Ratios <1 suggest net competition while ratios >1 suggest facilitation by alfalfa relative to the monoculture.

Year	**RY KAU/KKF**	**RY KAU/KKU**
2016	0.62	0.77
2017	0.89	1.01
2018	1.31	1.55
2020	0.32	0.60

Labeled Litter Incubation

Management Effects on Soil Organic Matter Formation

We predicted that the treatments with added nitrogen (KKF, KAU) would have greater accumulation of SOM due to more efficient microbial processing of litter substrates. We also hypothesized that SOM formation would be greatest in the KAU treatment since the decomposition of alfalfa residues could provide a source of N that would be available throughout the growing season, alleviating the need for N-mining and reducing N-loss which could result in greater C accumulation. We found no meaningful management effects (P=0.17, F=1.87, df=2) on the distribution of SOM in soil fractions, litter-derived SOM formation, or the amount of litter that remained undecomposed by the final sampling. There were slight differences at the 3-month measurement of bulk soil SOM-formation as discussed below (see Fig. 4.3). Since there otherwise were no detectable management effects, all the other results were averaged across management.

Litter Decomposition

All labeled litter achieved almost full mass loss by the end of the incubation as <6% of the initial litter C remained after 27 months of incubation (Fig. 4.3). The alfalfa roots exhibited the fastest decomposition, with only 2% initial litter C remaining by the first harvest at 3-months and 0.5% detected after 27 mo. Similarly, alfalfa shoots decomposed about twice as fast as Kernza shoots initially (*P*=0.001, *df*=68, *t*=5.68) but then followed a similar decomposition dynamic with 6% initial litter C remaining at the end of the experiment. Though slower to decompose initially, the proportion of Kernza roots remaining at the end of the experiment was similar to alfalfa roots. The amount of N retained in the litter ranged from 20-50% after 27 months (Fig. 4.3).

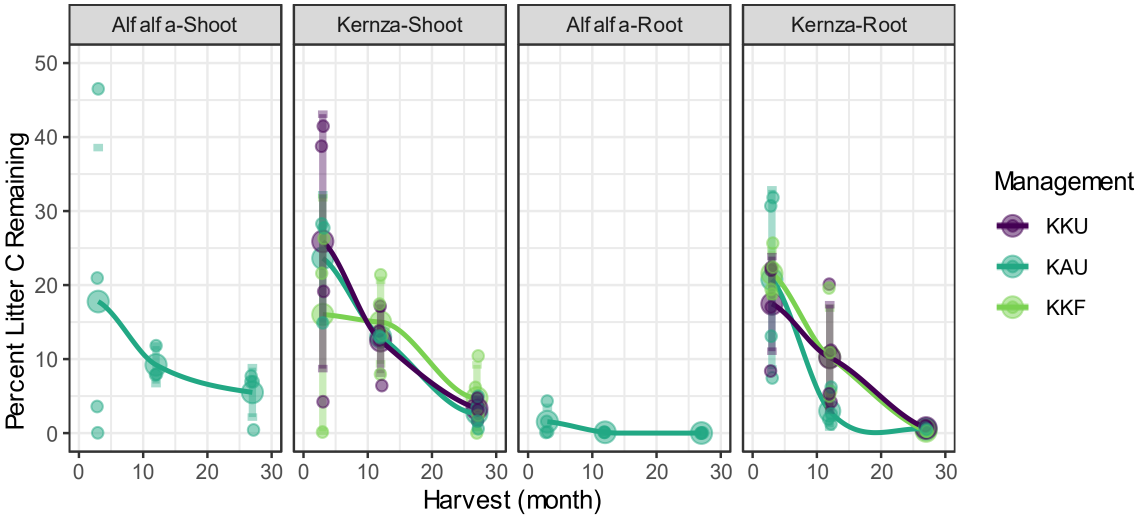

Figure 4.3. Litter carbon remaining in percent of the initial litter carbon at each harvest during the 27-month litter incubation by management. The larger dots represent the mean values of litter carbon remaining which was measured as the proportion of litter-derived carbon remaining at each time point for the reference labeled litter (either shoots or roots). Bars represent the standard error. The small dots represent the measured values for each soil collar (n=4 per litter type and management). Managements are as described in Fig. 4.1.

Litter-derived Soil Organic Matter Formation

Root and shoot litter showed distinct trends in how they were incorporated into soil over time (Fig. 4.4; P<0.001, F=6.2, df=2). Root-litter of both Kernza and alfalfa, which was mixed into the soil matrix, quickly contributed to soil C and N with 28% $^{+}/_{-}$ 3 *SE* of root-C and 57% $^{+}/_{-}$ 4 *SE* of root-N recovered in SOM by month 3. As decomposition progressed, root-derived C and N were lost from the soil with a tendency for the unfertilized monoculture (KKU) treatment to retain less root C (14% *vs.* ~21%) than the KAU and KKF plots (P=0.13, t=1.97, df=51).

The proportion of shoot-derived C and N recovered in bulk soil, in contrast, increased over the course of the incubation. At three months, 8% C and 21% N of the litter had become soil, and after 27 months the proportion rose to 11% for C and to 29% for N (Fig. 4.4; Table 4.4). The retention of shoots was less efficient than for roots as 35% more root material were recovered in bulk soil than shoots. While the preferential retention of root-litter C and N was evident in the KAU and KKF management at the end of the incubation ($P \leq 0.02$, $t \geq 2.5$, df=51), there were no differences in the amount of root-C in KKU (P=0.6) as compared to the respective shoot-litter.

Approximately 33% $^{+}/_{-}$ 3 *SE* more C and 27% $^{+}/_{-}$ 5 *SE* more N was recovered in bulk SOM from Kernza litter than alfalfa. These differences were chiefly in the root-C which was consistently less from alfalfa than Kernza throughout the incubation while the recovered root-N and shoot-C were similar between Kernza and alfalfa until the final harvest (root P=0.006, t=2.94, df=32; shoot P=0.07, t=1.83, df=32 for overall differences). By the end of the incubation, alfalfa roots and shoots were more similar in terms of C found in bulk soil (25% difference between tissue types) than Kernza roots and shoots (47% difference).

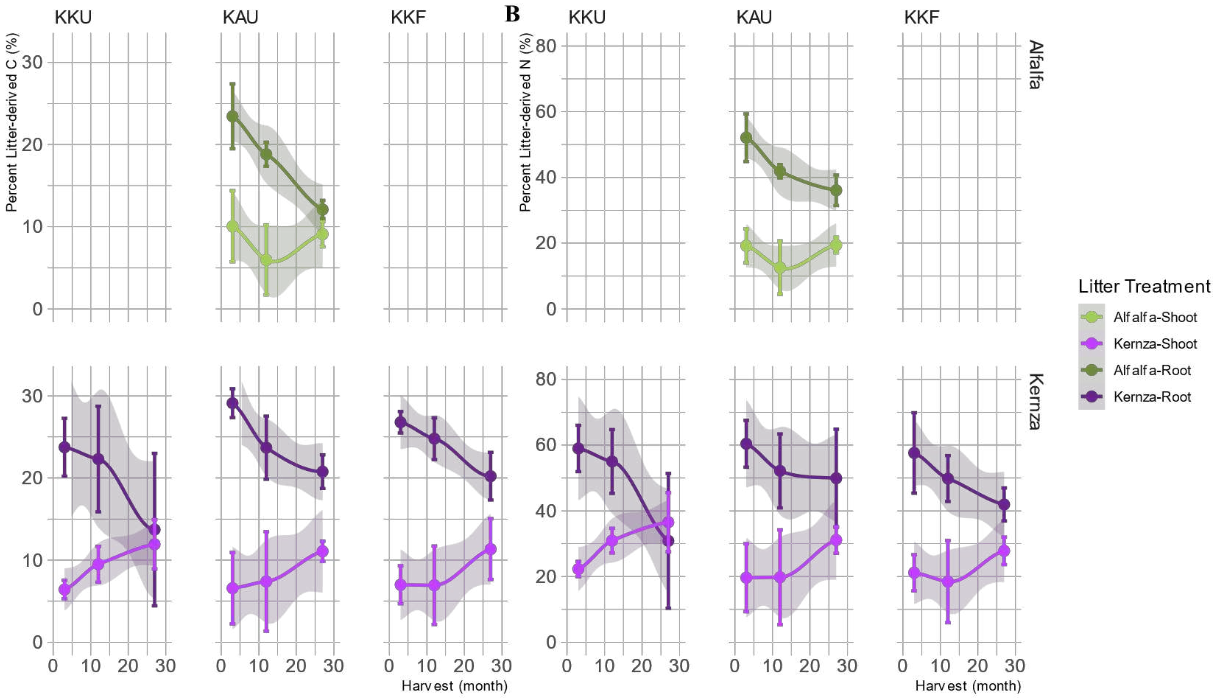

Figure 4.4. Percent litter-derived (A) carbon and (B) nitrogen for the bulk soil by litter treatment and management over time. Dots represent mean values for each litter type and bars represent standard error. The line is a LOESS regression curve fit to capture the 95% confidence interval around the mean. Management categories are as described in Fig. 4.1.

Dynamics of Soil Organic Matter Fractions

Distribution of SOM across fractions during the incubation

At the start of the incubation (3 mo), collars with added Kernza litter had ~2% (+/- 0.9% *SE)* greater proportion of MAOM than both alfalfa and control treatments (*P*=0.005, *df*=410, *t*=3.16). After 27 months, the added litter treatments had ~2% (+/- 0.9% *SE)* more oPOM than the controls (*P*<0.03, *df*=410, *t*=2.60). Surprisingly, the proportion of fPOM was stable across time and litter treatments with no increase in the collars with labeled litter (*P* ≥0.93). The alfalfa treatments consistently had lower proportions of MAOM, though the differences were slight (*P*≥0.08 3 mo; *P*≥0.48 12 mo; *P* ≥0.17 27 mo).

The control samples were the only treatment that had significant changes to the distribution of SOM over time. The mixed (disturbed) collars had a 4% $^{+}/_{-}$ 0.9 *SE* decline in oPOM over time ($P<0.0001$, $df=384$, $t=4.68$) and a proportional increase in MAOM ($P=0.0004$, $df=384$, $t=3.84$). The undisturbed control collars had a decrease in MAOM by ~2% $^{+}/_{-}$ 1.0 *SE* between the first and last harvest ($P=0.07$, $df=384$, $t=2.19$).

SOM Fractions Enrichment Dynamics

The $\delta^{13}C$ and $\delta^{15}N$ enrichment of SOM fractions show the contribution of litter-derived C and N to the soil fractions over time (Fig. 4.5). There were no differences between litter types for $\delta^{13}C$ enrichment of MAOM ($P\geq0.3$) over time, though initially alfalfa had higher $\delta^{15}N$ in MAOM than Kernza ($P\leq0.03$, $df=201$, $t\geq2.78$).

Root litter, especially for Kernza, was quickly incorporated into the oPOM and fPOM soil fractions (Fig. 4.5), with higher ^{13}C in fPOM initially and in oPOM after 27 months for Kernza than for alfalfa (3 mo: oPOM $P=0.8$, fPOM $P=0.02$, $df=165$, $t=2.35$; 27 mo: oPOM $P=0.04$, $df=193$, $t=2.02$; fPOM $P=0.2$).

Litter-derived SOM fractions formation

The trends apparent from the $\delta^{13}C$ and $\delta^{15}N$ enrichment explain the litter-derived C in the soil fractions over time (Fig. 4.5). Roots primarily contributed to fPOM initially (Fig. 4.5, 4.6), and over the course of the 27 months, all of the litter-derived root-C fractions decreased (-3% MAOM*;* -4% oPOM; -8% fPOM $^{+}/_{-}$1 *SE*; $P\leq0.02$, $t\geq2.8$, $df=160$). In contrast, shoot litter initially primarily contributed to the MAOM-C fraction (3% $^{+}/_{-}$ *0.8 SE* for alfalfa and 4% $^{+}/_{-}$ *1 SE* for Kernza; Fig. 4.5, 4.6). After 27 months, the litter-derived C from shoots increased 3-4% $^{+}/_{-}$ *1*

SE for all fractions (*P*<0.01, t≥3.63, df=160).

Kernza and alfalfa litter showed similar trends with the main differences being a more complete decomposition of alfalfa than Kernza roots (Fig. 4.6, 4.7) and a greater proportion of Kernza root litter contributing to SOM (Table 4.4, Fig. 4.7). Kernza litter contributed to POM to a greater extent than alfalfa (Table 4.4; Fig. 4.6, 4.7) (30% more fPOM; 42% more oPOM) by the final harvest. Noticeably, the amount of litter-derived MAOM-C and N was similar throughout the incubation for Kernza and alfalfa (P>0.2) (Table 4.4). The estimates of litter-derived C based on the sum of measured fractions and bulk soil were similar throughout the experiment (*only final harvest data shown*; Table 4.4).

Table 4.4 Litter-derived carbon (C) and nitrogen (N) by litter type and soil fraction for the final harvest at 27 months. fPOM stands for free particulate organic matter; oPOM for occluded particulate organic matter; MAOM for mineral-associated organic matter, Cumulated Fractions is the sum of the three measured soil fractions. Values in parentheses are the standard error.

Plant	**Litter Type**	***n***	**fPOM**	**oPOM**	**MAOM**	**Cumulative Fractions**	**Bulk**
Litter-derived C in %							
Alfalfa	Shoot	4	2.6 (0.8)	3.0 (0.4)	4.4 (0.2)	10.0 (1.5)	9.1 (0.8)
	Root	3	0.7 (0.3)	3.5 (0.5)	6.4 (0.3)	10.6 (1.1)	12.1 (0.6)
Kernza	Shoot	12	3.6 (0.8)	3.9 (0.4)	4.4 (0.2)	11.9 (1.4)	11.4 (0.8)
	Root	12	6.1 (1.1)	6.8 (0.5)	8.3 (0.3)	21.1 (1.8)	18.2 (1.8)
Litter-derived N in %							
Alfalfa	Shoot	4	4.1 (0.8)	4.5 (0.5)	9.0 (0.4)	17.6 (1.7)	19.4 (1.2)
	Root	3	2.3 (0.7)	6.6 (1.1)	20.2 (1.3)	29.1 (3.2)	36.1 (2.4)
Kernza	Shoot	12	6.5 (1.1)	6.7 (0.5)	13.3 (0.8)	26.5 (2.4)	31.8 (2)
	Root	12	6.9 (1)	8.1 (0.9)	24.5 (1.4)	39.5 (3.4)	40.9 (4.6)

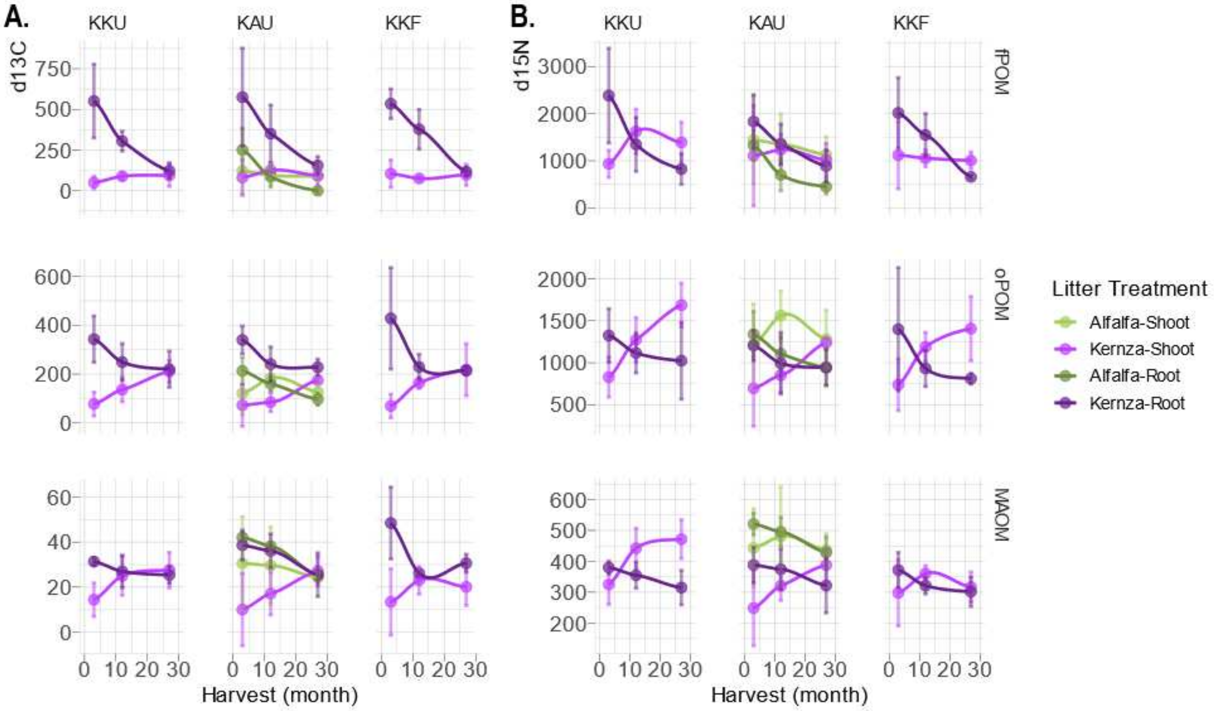

Figure 4.5. Isotopic enrichment of each soil organic matter fraction over 27 months based on the (A) δ^{13}C and (B) δ^{15}N by litter treatment. Dots represent mean values for each litter type and bars represent standard error. The line is a LOESS regression curve. Management categories are as described in Fig. 4.1. MAOM is mineral-associated organic matter (<53 µm); oPOM (≥53 µm) is occluded particulate organic matter; fPOM is free particulate organic matter (≥53 µm).

Estimates of SOC formation from Kernza and alfalfa residue by management

The estimated new SOC formation rates from annual crop inputs by management ranged from 47 to 79 Kg C ha^{-1} yr^{-1} for the 0-10 cm soil depth, with KKU having the smallest and KAU the largest, owing to the return of alfalfa shoot litter to the soil as residue (Table 4.5). As the Kernza shoots have the greatest biomass, they are predicted to have the greatest potential SOC accrual, though they are less efficiently converted into SOC. Owing that some of the aboveground biomass falls onto the soil during the growing season, we further estimated potential SOC formed from the surface litter based on the average litter density measured in the field (Table 4.2; 412 g m^{-2}) Using the same equation as for the shoot litter (Eq. 3), we estimate surface litter would increase the SOC accumulation rate by 45%. Similarly, our estimate of the root biomass from 10-100 cm (not measured in our study), suggests that an additional 48% of what might accrue in the surface (0-10 cm) soils could accrue from root decomposition at deeper depths (10-100 cm).

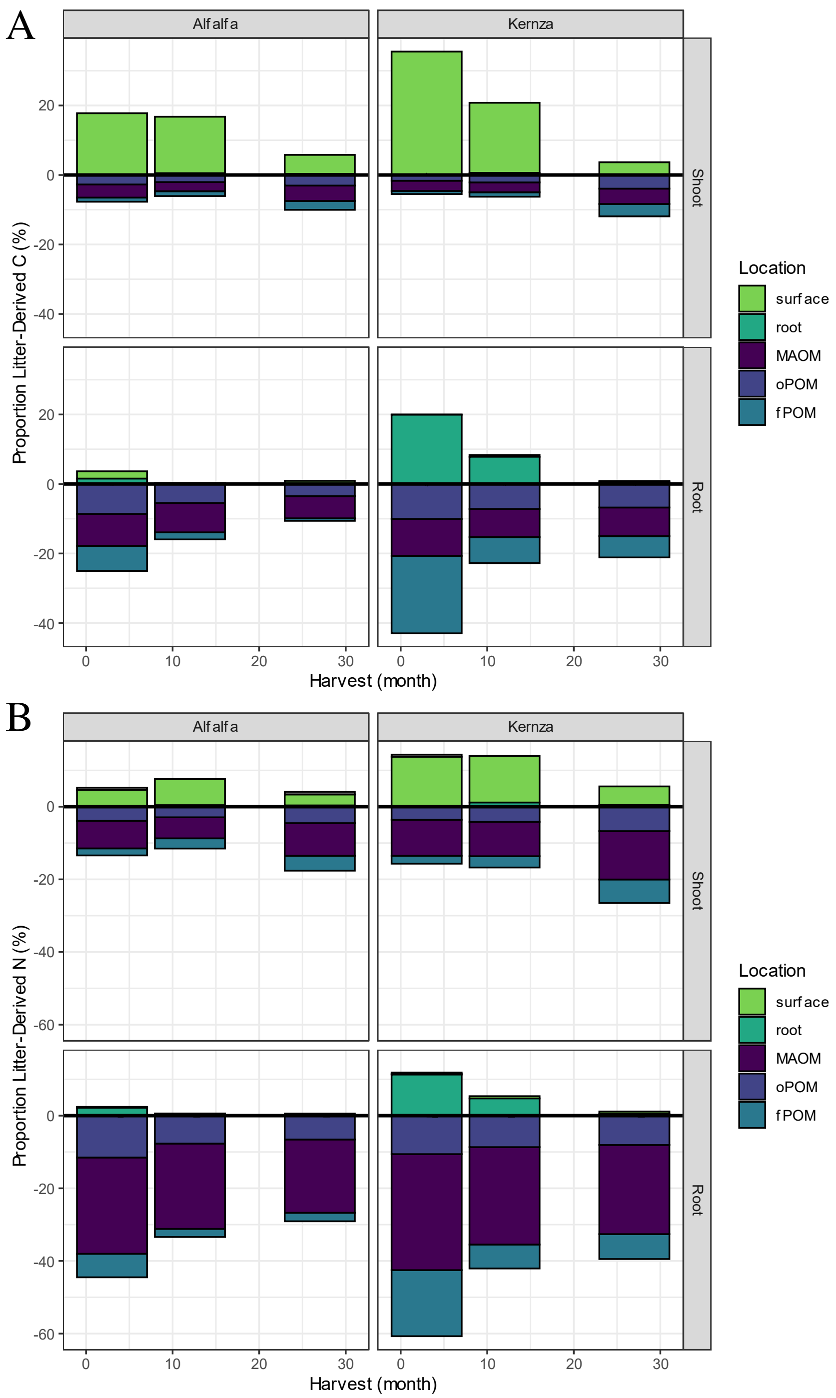

Figure 4.6. Proportion of litter-derived soil (A) carbon and (B) nitrogen in each measured soil and litter fraction over 27 months by litter treatment. Bars represent standard error. Root and surface refer to those respective litters in the soil collar; MAOM is mineral-associated organic matter (<53 µm); oPOM (≥53 µm) is occluded particulate organic matter; fPOM is free particulate organic matter (≥53 µm).

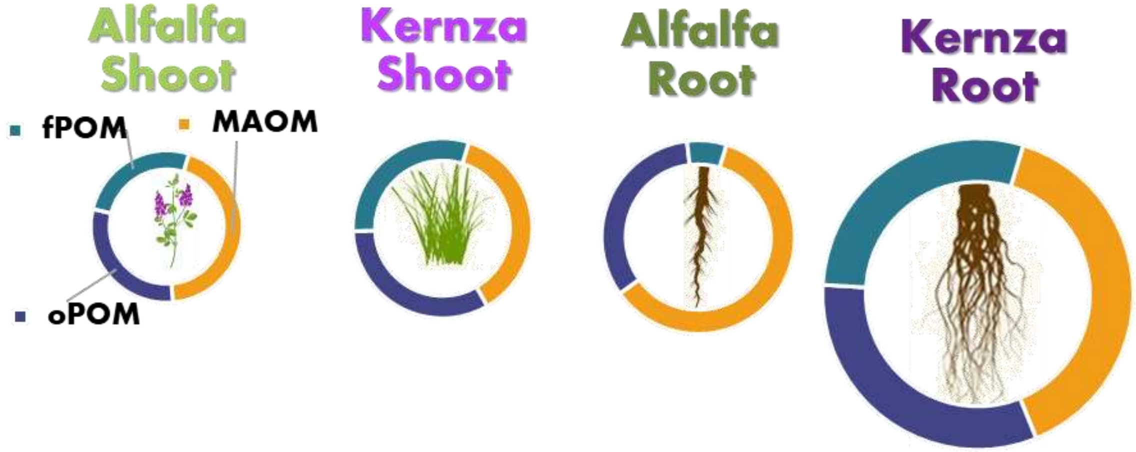

Figure 4.7. Proportion of litter-derived soil carbon by fraction for each tissue type at 27 months. Circles are proportionate to the quantity of litter-derived C formed (Table 4.4). MAOM is mineral-associated organic matter (<53 µm); oPOM (≥53 µm) is occluded particulate organic matter; fPOM is free particulate organic matter (≥53 µm).

Table 4.5 Estimated litter-derived C based on average aboveground net primary productivity (ANPP) 2016-2020 and labeled-litter SOM formation from this experiment in Salina, KS on silt loam soil as described in Methods. The surface litter amount was based on average values measured in the field in May 2019 and the average C concentration over the entire field. Root biomass 10-100 cm was estimated based on the average of measured values of root mass in Sprunger et al. (2018b) which ranged from 1005 to 2015 kg ha^{-1} over four years. Soil formation rates from each tissue type were assumed to be the same as was measured 0-10 cm. Standard error is roughly 10 Kg C ha^{-1} yr^{-1} based on the variability of the productivity. Shaded cells (Kernza shoots) were not included in the estimate of SOC formation with the assumption that they would be removed as part of standard management.

Management (0-10 cm)	**Tissue**	**Mean NPP** *(2016-2020)*	**C**	**SOC formation efficiency**	**Est. Contribution to Soil**	**Total New SOC**
	Unit	*Kg ha^{-1}*	*g C Kg^{-1} Plant*		*Kg C ha^{-1} yr^{-1}*	
Unfertilized Kernza Monoculture (KKU)	Shoots	6,160	418	0.11	129.3	105.8
	Roots	4,620	434	0.21	105.8	
Alfalfa intercrop (KAU)	Shoots	1,740	437	0.10	76.0	176.9
	Roots	1,305	430	0.11	14.9	
Kernza intercrop (KAU)	Shoots	5,078	420	0.11	241.0	
	Roots	3,808	428	0.21	86.0	
Fertilized Kernza Monoculture (KKF)	Shoots	8,315	420	0.11	394.6	145.1
	Roots	6,236	441	0.21	145.1	
Avg all Management	Kernza Surface Litter	2,150	254	0.11	61.7	
	Roots 10-100 cm	1510	441	0.21	70.3	
Fertilized Monoculture (KKF) Including Surface Litter and roots 0-100 cm						277.0

4. Discussion

Intercrop maintains SOC on par with fertilized treatment; Soil N stocks decline in all managements

The management practices initiated in the fall of 2015 led to different initial conditions of the soil (Fig. 4.1). The elevated soil N stocks in the fertilized treatment (KKF) imply that this management had the highest rate of N inputs and likely have the most N available for the Kernza crop as Kernza in this treatment had the lowest C:N and highest N-content of the plant tissues and HWE material (Table 4.1). Accordingly, the estimated N-fixation from alfalfa in KAU likely only exceeded the fertilization rate of KKF (100 kg N ha^{-1} yr^{-1}) in 2018.

The decline in ANPP and persistent negative N balance (Fig. 4.2) suggest N limitation early on after establishment; the rebound of ANPP following 2020 in the KKU and KKF management may additionally point to intraspecies competition limiting productivity if, after the 2018 drought, there was a significant root turnover alleviating competition and potentially provisioning greater N from mineralization of the root tissues in subsequent years. After three months, roughly 40% of root-N in this experiment was mineralized with the total mineralization increasing to 50-70% after 27 month. KKU plots had the greatest mineralization rate and KAU the lowest, suggesting tighter nutrient recycling in the treatments with greater N. The decline in productivity of Kernza stands has been frequently observed in other studies (Jungers et al., 2017; Hunter et al., 2017; Crews et al., 2022) and has been attributed largely to N-limitation.

The intercrop plots followed a replacement design meaning that a row of Kernza was replaced by a row of alfalfa in the KAU plots relative to the KKU and KKF. As there are fewer Kernza plants per area in KAU, we would expect reduced Kernza biomass due to this design, so it is not surprising that the monocultures often had higher ANPP (Fig. 4.2). That the ANPP in

KAU was >50% than the monoculture plots (Fig. 4.2A) indicates reduced competition overall. The relative yield ratio, which is the ratio of the productivity of the intercrop to the monoculture were <1 in all but 2018 compared to KKF, indicating that alfalfa may have had some net competitive effects on Kernza growth, though facilitated Kernza in 2018 (Table 4.3). Several studies of Kernza-alfalfa intercrops have found net competitive interactions in the first few years of the experiment (Tautges et al., 2018; Casamitjana, 2021; Crews et al., 2022), though interestingly net facilitation beginning around year 4 or 5 (Tautges et al., 2018; Crews et al., 2022). Since the alfalfa had disappeared from the plots by the 5th year after establishment due to selective gopher infestation, the intercrop in this experimental plot did not achieve that. The higher overall ANPP and RY>1 in 2018 suggest that the alfalfa did facilitate Kernza growth during that drought year, though productivity was low across all plots.

Intercrops of grass and legumes including Kernza and alfalfa (Li et al., 2019; Crews et al., 2022) often have 15-30% greater productivity and yield resilience compared to monocultures (*summarized in* Renard and Tilman, 2021). The intercrop in this study may have underperformed compared to previous studies as, at the point where the effect of alfalfa on Kernza shifts from competitive to facilitative, much of the alfalfa had died.

We estimated that the inputs from fertilizer (100 kg N ha^{-1} yr^{-1}) in the KKF treatment were unlikely to replace the N exported from the Kernza ANPP in all but one year (2018) when drought limited productivity (Fig. 4.2B). The KAU treatment similarly likely has a net N export from Kernza especially in the establishment year (2016) when alfalfa N_2-fixation and decomposition was likely limited. The initial high productivity of KKU in the first year reflect the underlying high fertility that is able to support plant growth despite declining N stock (Fig. 4.1).

These potential shortfalls of N imply that soils under all managements in this study could have declining N stocks, though under the KKF and KAU soil N would decline more slowly as N added from urea fertilizer or N_2-fixation could meet or exceed the N-demand and export at least some years. The net trend of negative N balance may limit the capacity of these treatments to accrue SOC, particularly as MAOM-C which has a lower C:N (< 15) (Crews et al., 2016). A net gain of 0.3 Mg C ha^{-1} over 5 years (Table 4.5) would require at least a positive N balance of 20 kg N ha^{-1} yr^{-1} (Crews et al., 2022). This threshold was likely met in 2018 when productivity was limited, potentially restoring some of the soil N stock.

Contrary to our hypothesis, KAU did not have greater proportion or stock of MAOM-C or N than KKF. Instead, the intercrop had lower N stocks overall (Fig. 4.1B) and a lower C:N ratio than the monoculture management (Table 4.1). The lower N stock and C:N in the soil imply that the organic-N inputs from alfalfa may promote greater microbial activity and turnover as the SOM has a greater microbial signature, thus there may be an enhanced *in vivo* pathway of SOM formation (Liang et al., 2017). The accelerated turnover may result in greater C losses, however, and negate new SOC formation even if C use efficiency is higher. Despite the presumed faster turnover of alfalfa residue and microbial biomass in the KAU plots compared to monoculture, the higher Kernza shoot C:N (Table 4.1) also implies that the intercrop has not met Kernza's N-demand as well as the fertilizer (KKF). The seeming lack of benefit of alfalfa to the Kernza in KAU may be particular to alfalfa as a legume. Studies comparing the N-transfer from legumes to grasses have found that despite the high N-fixation rates of alfalfa [90-386 kg N ha^{-1} (Peoples et al., 1995)], other legumes such as clovers share a greater amount of N with grass species over two years (Louarn et al., 2015; Thilakarathna et al., 2016). This may be due to the greater proportion of structural components in alfalfa tissues, in particular its woody tap roots, compared to the fibrous and shallower roots of clover species. For maximizing N-synchrony between Kernza and a legume, a more nuanced management may be needed such as using multiple species of legume intercrops, combining legumes with additional N amendments when establishing a new stand, and/or seeding Kernza into established legume stands as proposed by Crews et al. (2022).

Roots and shoots decompose over different timescales and roots are preferentially retained

There were few management differences in how labeled litter decomposed and were converted to SOM. This may reflect the impact the soil collars themselves may have had on the decomposition, shielding the soil in the collar from potential management effects. The collars did have holes to allow for roots to enter the soil within the collars, though the root content within collars decreased over time for the samples without added root litter. This suggests that the effects of the management may have not been captured inside the collar. Thus, though there were known differences by management based on the SOC stocks at the start of the experiment and the contrasting productivity and N input rates, the discussion will focus on differences between litter types rather than management.

The decomposition of the labeled litters was near complete by the end of the 27-month incubation regardless of litter type or placement, similar to other studies with labelled litter incubated with direct contact with the soil and elements (e.g. not in a litter bag) (Cotrufo et al., 2015; Haddix et al., 2020). The decomposition of all litter in this experiment occurred faster than similar experiments with labeled grass litter (Soong and Cotrufo, 2015; Leichty et al., 2020). Beginning the experiment at the start of the growing season (May) and cutting the litter to 2 cm lengths likely accelerated the decomposition. The apparent relatively slower decomposition of

Kernza and alfalfa shoots in the KAU plot as compared to the other plots might have been due, in part, by gophers' disturbance burying several collars in this treatment between the first and second harvest. The collars that were buried by gophers had substantially higher undecomposed litter than average (e.g. 93% vs. <15% undecomposed for buried and unaffected collars). Aside from these anomalies, decomposition occurred most rapidly for roots especially in the intercrop (KAU) management

The litter chemistry was universally high in N compared to the plants growing in the field as the C:N ratios of labelled litter ranged from 9 (alfalfa shoots) to 24 (Kernza roots) whereas plants in the field had C:N from 15 (alfalfa shoots) to 55 (Kernza roots) (Table 4.1). The low C:N as well as tendency for lower AUR/N compared to field-grown plants suggest the labeled litter, and especially that added through soil disturbance in the collars, may have decomposed faster than native litter (Melillo et al., 1982; Freschet et al., 2013). The lower C:N and faster decomposition may ultimately have led our study to underestimate the potential of Kernza to accrue SOC, especially in POM (van der Pol et al., 2022), as many studies have found that low C:N coupled with rapid decomposition leads to little or no SOC accumulation (Vesterdal et al., 2008; Mueller et al., 2015; Xia et al., 2018).

Due to its placement in the soil matrix (0-10 cm), the root litter was incorporated into the soil and promptly formed SOM; shoot litter, in contrast, became SOM over time as the litter underwent fragmentation and microbial processing (Fig. 4.4). Similar studies have noted that incorporating labeled litter into the soil results in more efficient SOM formation (Mitchell et al., 2016; Leichty et al., 2020). The placement of the labeled litter had a greater effect on its incorporation into SOM than did the litter chemistry. Differences in the labelled litter chemistry were less than expected based on the field-measured values of contrasting litter types (Table 4.1).

Thus, our experiment was not a robust test of how litter chemistry influences SOM formation. The labeled Kernza roots had a AUR/N ratio 5 times greater than the shoots compared to a difference of 1.7 times higher AUR/N roots:shoots for alfalfa (Table 4.1). The similarity of alfalfa roots and shoots may explain, in part, why the alfalfa roots decomposed so completely and quickly (Fig. 4.4).

Both Kernza and alfalfa root C was incorporated into bulk SOM to a greater extent (+18% for alfalfa and +63% for Kernza) than the shoot C. These findings are consistent with the results of experiments (Puget and Drinkwater, 2001; Freschet et al., 2013; Fulton-Smith and Cotrufo, 2019) which show that root-C is preferentially retained in soil and may be the main source for SOM formation (Rasse et al., 2005; Poirier et al., 2018; Sokol and Bradford, 2018). In contrast to many prevailing frameworks for root-C retention which base the persistence of root C on its slower decomposition (Puget and Drinkwater, 2001; De Deyn et al., 2008; Hobbie, 2015), however, we observed rapid root decomposition and near total mass loss after 2 years. By the end of the incubation, roots had higher retention in the soil despite similar amount of mass remaining than shoot litter, suggesting they were more efficiently retained. The simplest explanation for this enhanced SOM formation from roots is that they were in greater contact with the soil particles and more efficiently stabilized likely due to the combined effects of decreased distance from mineral surfaces and increased exposure to soil microbes (Sokol and Bradford, 2018) facilitating some MAOM formation as well as occlusion within aggregates as oPOM. Unlike other studies that mixed litter in with the soil, we found no evidence that the added litter promoted losses of native soil C which exceeded the amount of C added (Mitchell et al., 2020).

Legume residues did not promote greater quantities of MAOM-formation; Kernza contributed both to POM and MAOM C

We hypothesized that the greater concentration of extractable and weak-acid soluble components and N in the alfalfa tissues would lead to increased efficiency of SOC stabilization as MAOM, and that the greater proportion of structural components in roots and Kernza tissues would result in greater POM formation. As hypothesized, Kernza contributed to POM to a greater extent than alfalfa (Fig. 4.7). Contrary to our hypothesis, however, labeled litter from Kernza tissues were 39% more likely to become SOM than the alfalfa, and both crops formed similar quantities of MAOM-C after 27 months (Table 4.4). The greater contribution of Kernza to SOM was driven by the fact that ~9% more of Kernza roots remained as POM at 27 months than alfalfa.

Despite the greater overall formation of SOC from Kernza, the relative proportion of SOC that formed MAOM-C from alfalfa tissues was higher than that of Kernza (Table 4.4; Fig. 4.7). While the majority of Kernza-derived SOC became POM, the majority of alfalfa-derived SOC became MAOM. These findings are in line with our hypothesis that the tissues with more metabolic and digestible molecules would preferentially become MAOM-C (Cotrufo et al., 2013) despite that they did not lead to overall greater quantities of MAOM. Compared to other studies with field-incubated litter, proportions of Kernza incorporated into SOM as MAOM and POM were similar to labelled Big Bluestem (*Andropogon gerardii*) aboveground tissues that were incorporated into the soil (0-10 cm) (Leichty et al., 2020). That study found a similar ratio of litter-derived POM:MAOM-C (~60:40) and 27% of the original litter as SOM after 12 months (Leichty et al., 2020). A study contrasting root and shoot tissues of sorghum (*Sorghum bicolor*) found that 27% of root tissues and 19% of leaves became SOC after 19 months with roughly 5%

of root-C as MAOM and 8.5% of leaves as MAOM (POM:MAOM ratio 65:35, Fulton-Smith and Cotrufo 2019). As these previous studies were of shorter duration, the amount of litter-derived Kernza in this study seems on par if not greater than the litter-C retention of these other perennial grass species. The Kernza roots in this study also formed almost twice as much MAOM-C as sorghum root tissues in this previous study (Fulton-Smith and Cotrufo 2019), possibly due to the relatively high C:N and low AUR/N of the labelled litter in this experiment (Table 4.1, 4.4).

Our hypothesis that Kernza, having more structural components, would contribute more to POM than alfalfa was supported (Table 4.4; Fig. 4.6). The differences between the Kernza and alfalfa labeled litter material are less than what we expected given the tissue chemistry of the plants growing in the field (Table 4.1).

Due to the chemical differences in the field and greenhouse-grown litter, our study may under-estimate Kernza's contribution to POM and overestimate that to MAOM. That would be consistent with the findings in Ch. 3 (van der Pol et al., 2022a) where I observed variable effects of Kernza on MAOM-C by site but significantly increased POM compared to annual crops. Greater structural content and decomposition rate may also increase the proportion of POM occluded in aggregates as macroaggregates have been found to contain greater quantities of lignin-like compounds in cropland and grassland soils (Panettieri et al., 2017).

Estimating the capacity for Kernza to sequester soil carbon

One potential benefit of perennial grains is the ability to accrue SOM especially if they replace annual crops (Ryan et al., 2018). Our estimates are that roughly one fifth of the Kernza root biomass became SOM in the ratio of 3:2 POM:MAOM. In contrast, only ~12% of Kernza aboveground tissues became SOM after two years with only one third of that sequestered as MAOM-C (Table 4.4).

We used the proportion of litter-derived C and ANPP by management (Fig. 4.2) to estimate the amount of SOC accrual we might expect to see over time (Table 4.5 for bulk soil; *accrual rates for POM and MOAM separately not shown but calculated the same way*). On average, we would expect similar amounts of SOC to form under KAU as KKF (Table 4.5), though with slightly more SOC formation predicted for the intercrop. Given the tendency for more alfalfa to remain as MAOM than POM, we would expect slightly more MAOM to accumulate under KAU than KKF (76 vs. 57 $^{+}/_{-}$ 10 *SE* kg C ha^{-1} yr^{-1}), but these differences are slight and unlikely to be detectable over short timescales. The estimates are also largely dependent on the ANPP which varies one order of magnitude annually; using the mean values for the estimates does not capture this variability well.

Regarding how N management influences SOM formation and the potential to increase SOC, we found negligible effects. There was some evidence that, four years after these plots were established, the KKF management had higher SOC than the unfertilized monoculture (KKU) (Fig. 4.1). Though we might expect the KAU treatment to retain more SOC based on a greater proportion of the ANPP being left in the field each season (Table 4.5), we did not observe evidence of this.

The challenge with assessing the role of N management on SOC accumulation are the confounding effects of N and productivity. We hypothesized that the KAU might have more efficient SOC formation and thus accrual as may have been observed in Ch. 2 (van der Pol et al., 2022b), but the overall lower productivity of this management compared to KKF may mask potential increases in carbon use efficiencies. Other studies that examined the role of plant diversity and legumes on increasing SOC have found no relationship with SOC accrual and legumes (Carranca et al., 2009a; Gentile et al., 2011; Mueller et al., 2015; King and Blesh, 2018; Chen et al., 2018). A study that used isotope tracers to assess soil C dynamics found that plant diversity (but not legumes as a functional group) promoted SOC accrual primarily by increasing productivity as net primary production (NPP) and slowing the decomposition of the plant tissues (Lange et al., 2015). The legume intercrop in this study supported higher ANPP only one out of four years, but the legume tissues decomposed rapidly compared even to the relatively highly decomposable labeled Kernza litter (Fig. 4.3). As with the conclusion of Carranca et al., (2009), the alfalfa material may be decomposing too rapidly and completely to have a lasting impact on the microbial community biomass or aggregate C that could lead to enhanced SOC accrual. A meta-analysis comparing SOC sequestration and N losses based on C:N of residues found that only residues with C:N > 30 supported SOC accrual (Xia et al., 2018). Along with that finding, another study found that high quality litter could have a counteracting effect on SOC as the rapid and complete decomposition of high quality litter meant there was scarce structural components remaining which resulted in lower overall SOC values (Mueller et al., 2015).

Our estimates (Table 4.5) are lower than other measures of SOC gains under perennial production (130 – 1700 kg C ha^{-1} yr^{-1} in Crews and Rumsey, 2017), though our estimates are only for 0-10 cm. Kernza roots likely have an additional 0.8-1.6 Mg root biomass ha^{-1} at depths from 20 to 100 cm (Pugliese et al., 2019), the total SOM accumulation from an established Kernza stand is likely 0.05 – 0.09 Mg C ha^{-1} yr^{-1} for depths 10 – 100 cm (Table 4.5). Considering the additional C from roots 10-100 cm and the likely contribution of surface litter to SOC, the estimated accumulation rate based on the average KKF productivity is 0.11 Mg C ha^{-1} yr^{-1} $^{+}/_{-}$ 0.01 *SE* 0-100 cm which would be similar to the lowest estimate by Crews and Rumsey (2017).

The labelled litter used in this experiment also had higher proportions of metabolic compounds which may lead to us underestimating SOC accrual as a significant mechanism for SOC gain from Kernza may be as POM through the physical and ex vivo formation pathways (Cotrufo et al., 2015; Liang et al., 2017). Additionally, our assumption of 50% root turnover each year does not account for the accumulation of root biomass over time that may make a substantial contribution to SOC formation. The true root turnover rate may be closer to 75% as suggested in studies using eddy covariance towers (de Oliveira et al., 2018). Finally, as the C:N and structural components of roots increase with depth (Wang et al., 2016; Poirier et al., 2018), the proportion of SOC accrued as POM would likely increase with depth as has been found in Ch. 3 for Kernza (van der Pol et al., 2022a) and generally for root-derived SOC (Gill et al., 1999).

These accrual rates are likely underestimates of total SOC sequestration for a couple reasons. First, we did not account for the contribution of rhizodeposition from living roots to SOC. Crop and grass species roots exude an estimated 7 to 11% of gross primary production into the soil, of which 3-5% is retained for longer periods of time (Pausch and Kuzyakov, 2018). The quantity of rhizodeposition is difficult to estimate. Plant species vary significantly in the amount, timing, and chemical composition of their exudates as these perform a variety of functions for the plant such as nutrient scavenging and responding to environmental or pathogen stress (Sasse et al., 2018). Root exudation is likely to occur in the A horizon where the microbial community and nutrient concentrations are highest, rhizodeposition could be a significant source of MAOM-C (Villarino et al., 2021). While some studies have found rhizodeposition may promote SOC loss through priming of native SOC (Huo et al., 2017; Jilling et al., 2018), a meta-analysis of rhizodeposition found that 75% of the belowground-derived MOAM-C was from rhizodeposits (Villarino et al., 2021).

Second, the low AUR/N and high N-content of all labeled litter may have resulted in unusually fast decomposition rates which could have led to decreased SOC stabilization (Mueller et al., 2015). The labeled litter with the highest AUR/N (Kernza root) also had the highest amount preserved as oPOM and overall (Fig. 4.6; Table 4.4), pointing to the combined importance of the structural and metabolic components being present in litter for efficient stabilization in soil.

The previous estimates of Kernza's ability to increase SOC have been observational studies compared to annual crops. A 4-year experimental treatment with variable N application rates found Kernza had increased above and belowground biomass (0-70 cm) compared to annual crops, but that there was no net change to SOC (Sprunger et al., 2018a). The study authors point out that in the sandy soils at the field site would have required a sizeable change to SOC (>15%) to detect a difference between the annual and perennial crop, however. Chapter 2 of this work, an observational study of working and experimental Kernza fields in Kansas, USA estimated Kernza accrued SOC at an average rate of 0.4 Mg C ha^{-1} yr^{-1} (0-100 cm) assuming similar starting SOC to paired annually cropped fields (van der Pol et al., 2022a). This estimate greatly exceeds the rates estimated here, potentially indicating the high fertility of the soils in this study compared to those in Ch. 2. Finally, an observational study monitoring net ecosystem exchange over Kansas-based Kernza fields estimated a 4-year average net C uptake rate of 3.7 Mg C ha^{-1} yr^{-1} (de Oliveira et al., 2018). This estimate includes plant tissues as well as any C conferred to the soil. The SOC accrual rates estimated in this study are significantly lower than previously estimated but given the mechanistic nature of this study and direct measurements of SOC in the same field rather than a paired design, these estimates could be more reliable. On the other hand, the unusually labile and N-rich nature of our labeled litter as well as the altered conditions imposed by the small PVC collars on decomposition could render these estimates significantly different than what might otherwise occur in the field.

5. Conclusion

Perennial grains have the potential to restore many ecosystem services hampered by annual agriculture including increasing SOC. Our results indicate that Kernza supports SOC accrual rates between 0.11-0.18 $^{+}/_{-}$ 0.01 *SE* Mg C ha^{-1} yr^{-1} primarily from root C in surface soils in the ratio of 3:2 POM:MAOM. Roughly 20% of root tissues C incorporated into SOM after 27 months with 8% as MAOM-C and 7% as oPOM-C, implying that 15% of root tissues C may be stabilized in the soil. We did not find evidence that intercropping with the legume alfalfa further enhanced SOC though it did sustain similar levels of SOC as the fertilized monoculture. Similar to other intercropping studies with alfalfa, we found evidence for competition with Kernza at the onset of the stand establishment and we estimated a negative N balance for the first couple of years for all management practices.

We found that the N management had little influence on the decomposition. Since our labeled litter differed in its chemical composition significantly from the field-grown plants , future similar studies should use caution when growing labeled plant materials for decomposition studies. Finding ways to promote similar plant growth outdoors would make the litter decomposition more realistic as greenhouse grown plants may be inherently more likely to contribute to the MAOM soil fraction and have accelerated decomposition rates.

Future studies should address management strategies and environmental conditions that support intercropping that improve the facilitation of the grain crop by the intercropped species and promote fine root growth and sustained microbial activity. Promising research directions include multispecies legume intercrops, livestock integration, and identifying management practices that sustain modest positive net N balance to promote efficient N cycling and SOC accrual.

Finally, given the importance of oPOM together with MAOM for SOM stabilization requires reframing the importance of higher litter quality in promoting overall SOC stabilization and including the role that higher AUR/N litter may have in oPOM formation. There may be a threshold of labile:structural components that optimizes SOC formation and protection. Too few structural components may result in litter that is too short-lived to remain in the soil for a significant time, while too little N coupled with too much structural material may result in very low carbon use efficiency and similarly little SOC formation. Experimentation with the proportions of the structural and labile tissues in soils with contrasting minerology and soil N could identify the relevant thresholds for optimizing microbial CUE and aggregation for long term SOC sequestration.

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CHAPTER 5: REMOVING BARRIERS AND CREATING OPPORTUNITIES FOR CLIMATE-RESILIENT AGRICULTURE BY OPTIMIZING FEDERAL CROP INSURANCE[6]

1. Climate change and agriculture

Cost of climate change

Increased temperatures and extreme weather events of the past decades are expected to worsen (Ray et al. 2015) with projected temperature increases of 1 to 3°C by 2050 (Hatfield and Takle 2014). While temperature increases might lengthen certain crops' growing seasons, the overall effect of climate change will increase crop failure and reduce yields (Lobell and Gourdji 2012; Wienhold et al. 2018). Many farmers purchase crop insurance managed by the U.S. Department of Agriculture (USDA) as protection from devastating crop loss, and taxpayers cover ~60% of premium costs for this Federal Crop Insurance Program (FCIP) (Bryant and O'Connor 2017). Under a 2°C warming scenario, economists estimate the tax-burden for payouts to increase by $3.7 billion (Tack, Coble, and Barnett 2018). Cost increases are exacerbated by the increasing value of insured crops, which leads to greater liability to taxpayers (Bryant and O'Connor 2017). Efforts to optimize FCIP are, therefore, economically important.

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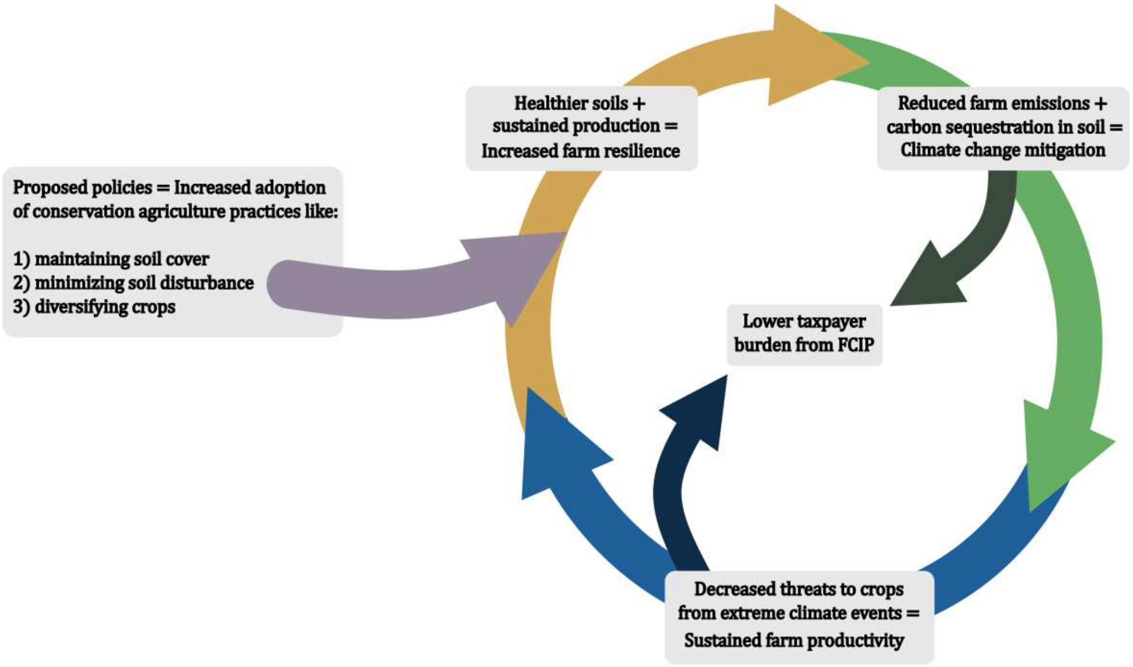

Figure 5.1: The proposed policy options would modify U.S. Department of Agriculture research funding and aspects of the Federal Crop Insurance Program (FCIP) to enhance conservation agriculture. Through a feedback loop, these changes would lead to food systems becoming more resilient to climate-induced extreme events, potentially lower costs of FCIP, and mitigate climate change through reduced greenhouse gas emissions and carbon sequestration.

Synergy between conservation agriculture and climate change mitigation

Agricultural practices and climate change are linked (Fig. 5.1). Farming practices such as monocropping, fallow, and tillage have degraded soils (Sanderman, Hengl, and Fiske 2017), making them more dependent on energy-intensive management and susceptible to crop loss (Tilman et al. 2002). The extreme climatic events that characterize climate change also threaten continued ability to cultivate crops (Hunter et al. 2017). Thus, there are clear feedbacks between agricultural practices and climate change.

Conservation practices, on the other hand, can rebuild soil health. For example, diversifying crops, maintaining soil cover, and minimizing disturbance can restore soil structure, facilitate nutrient cycling, and promote carbon sequestration (Robertson et al. 2018; Knowler and Bradshaw 2007). Specifically, the formerly grassland soils of the Northern Great Plains offer potential climate change mitigation because poor management has led to the depletion of more than half the original soil carbon (Kern and Johnson 1993), consequently providing the opportunity to rebuild soil carbon through conservation practices (Lal 2018). Incremental increases to soil carbon are a significant, affordable, and agronomically beneficial means of greenhouse gas (GHG) reduction (Chenu et al. 2019). Enhancing soil carbon can also increase crop yields (Oldfield, Bradford, and Wood 2019), farm resilience to extreme events (Bowles et al. 2020), and lower FCIP payouts (Kane et al. 2021). Thus, conservation agriculture is an effective nature-based solution to climate change because practices that increase soil health simultaneously mitigate climate change (Bossio et al. 2020).

Imperative for focusing on semi-arid region and legumes for diversification

Our recommendations focus on the semi-arid Northern Great Plains based on the compelling evidence of the benefits of conservation agriculture in this region and the historical practice of the USDA to implement reforms to FCIP after testing in a localized region (Hamilton 2020). This area frequently experiences drought and extreme temperatures, making it prone to FCIP payouts (U.S. Government Accountability Office 2015). As climate change is expected to expand semi-arid regions and intensify these events, farmers in other geographic regions will increasingly be faced with similar environmental challenges (IPCC 2019). Simultaneously, the funding and capacity of plant breeding programs in the U.S. have declined, forcing plant breeders to focus on maintaining infrastructure rather than diversifying crops (Coe et al. 2020). Only few crop breeding programs exist for legumes (Rubiales et al. 2015; Cernay et al. 2015).

Yet, legumes offer great potential to offset fertilizer use while enhancing soil health and carbon sequestration (Jensen and Hauggaard-Nielsen 2003; Peoples et al. 2009). We thus focus our recommendations on this region and specifically target legumes for regional crop development. If effective at reducing FCIP costs and improving agriculture resilience on a regional level, our recommendations could be scaled nationally.

Strategies and policy options to promote conservation agriculture

As climate change threatens agriculture and increases financial burdens to farmers and taxpayers, urgent modifications to FCIP and USDA research funding that promote nature-based solutions are imperative (Fig. 5.1). We suggest this be implemented as a pilot program due to the precedent that the USDA uses pilots to trial reform measures (Hamilton 2020).

We recommend three specific changes:

- Eliminate county-level fallow requirements;
- Fund research and market development for regionally-adapted legumes to provide suitable fallow alternatives; and
- Phase out single-crop, yield-based coverage and incentivize whole farm revenue protection.
- If adopted, these policies would reduce GHG emissions, improve soil health and farm income, and confer greater resilience to climate change.

2. Option 1: Eliminate county-level fallow requirements for wheat to promote soil health and crop diversification

In the early 20th century, agriculturalists advised farmers to stabilize winter wheat yields by implementing fourteen months of bare fallow to store water in the soil before planting (Peterson et al. 2020). Decades of research have shown, however, that the fallow does not store water efficiently (Nielsen and Vigil 2010). The rise of no-till farming, in which the crops are planted without plowing, has allowed many farmers to plant a productive crop annually without summer fallow periods, thus protecting soil from erosion, increasing soil carbon, and leading to greater overall yield and income (Peterson et al. 2020; Rosenzweig, Fonte, and Schipanski 2018). The adoption of more frequent and diverse cropping practices, however, has been hindered by FCIP policy that requires fallow prior to sowing wheat to insure the crop (Rosenzweig, Carolan, and Schipanski 2019). Fallow periods promote erosion, soil carbon and nutrient loss, and limit soil water-holding capacity. Thus, fallow periods can reduce farm resilience to extreme events (Rosenzweig and Schipanski 2019).

Farmers in Colorado and surrounding states already recognize the advantages of cultivating diverse crops and eliminating fallow: more than 0.5 million hectares reduced the frequency of summer fallow by incorporating more frequent short-season crops between 2008 and 2016, for example, by shifting to a wheat-corn-fallow rotation (Rosenzweig and Schipanski 2019). The reduction of fallow has resulted in an estimated 9% increase in grain production, an \$80 million increase in farm income, and a 3% reduction of annual GHG emissions from the Colorado agricultural sector—equivalent to emissions from 70,000 passenger vehicles (Rosenzweig and Schipanski 2019). Broader elimination of fallow is limited by FCIP whose coverage varies by county: 78% of eastern Colorado counties require fallow before planting wheat to be eligible for crop insurance. Removing the fallow requirement would promote more frequent and diverse crops with the potential to increase regional grain production by 25%, raise farm income by 26%, and almost completely offset GHG emissions caused by dryland (non-irrigated) grain (Rosenzweig and Schipanski 2019).

Advantages

- Removing the fallow requirement would provide greater farmer autonomy, allowing them to select the best strategies for their farm and climate. It would permit farmers to crop continuously—a practice that has been shown to benefit soil health, yield, and farm income—while concurrently mitigating climate change through reduced net GHG emissions and increased soil carbon sequestration (Jensen et al. 2012)
- Efforts to promote soil health can increase agricultural yield and therefore reduce taxpayer risk through FCIP
- Changes to existing policy requirements can be made at no cost of implementation

Disadvantages

- Fallow can enhance yield the year wheat is harvested, though it reduces yields averaged over a rotation cycle (Nielsen, Lyon, and Miceli-Garcia 2017).
- Additional barriers beyond FCIP influence grower adoption of conservation agricultural practices, so other policy changes will be required to achieve resilient agricultural systems.

3. Option 2: Fund research and market development for regionally-adapted legumes to provide suitable fallow alternatives and increase crop diversity

The variety of crops planted in the U.S. is decreasing (Crews, Carton, and Olsson 2018). From 1945 to 2007, the Corn Belt acreage devoted to corn and soybean increased from 29% to 80% (Claassen, Langpap, and Wu 2017). FCIP incentivizes planting a small number of commodity crops in excess of domestic need, exacerbating crop homogenization, depressing commodity prices (Bryant and O'Connor 2017), and making food systems vulnerable (Beckie et al. 2019). Suitable alternatives are urgently needed. Moreover, removal of fallow requirements, per Option 1, will further drive the need for suitable alternatives to fallow.

Historically, the emergence of a new crop is preceded by significant public investment in research, crop breeding (Cernay et al. 2015), and market development (Zander et al. 2016). For example, USDA-funded research led soybean yield to double since 1965 (Anderson et al. 2019), and USDA-developed corn varieties made it possible to plant in northern regions previously too risky to sow due to cold and dry conditions (Plant Breeding Working Group 2015) This research led to increased yields and nutrition and serves as the seeds utilized by private corporations today. In addition, other countries have successfully increased farm diversification and economic opportunity by investing in crop breeding and marketing (Maaz et al. 2018). Canada invested CAN$18 million in the 1970s to develop the edible oilseed canola, which resulted in CAN$1 billion in benefits to growers and a return on investment to taxpayers of over CAN$700 million (Brewin and Malla 2012). These investments have led to success, in part, because they developed crops for specific climate regions. Hence, we propose similarly scaled investments to research regionally-adapted pulse[7] crops for the U.S. Northern Plains.

Alternative crops could benefit local economies while conferring many ecological benefits from diversification (Peterson-Rockney et al. 2021). For this pilot program, legumes offer the greatest potential, as they have short growing seasons (minimizing water use), enhance nutrient cycling, boost subsequent crop yield, reduce reliance on fossil-fuel-based fertilizers, and provide a protein-rich food source (Stagnari et al. 2017; Zander et al. 2016; Crews and Peoples 2004). Additionally, many legumes could be bred for intercropping, which would allow for simultaneous forage[8] and grain production (Wahbi et al. 2016; Li et al. 2001; 2014) and have the potential to enhance carbon sequestration (Drinkwater, Wagoner, and Sarrantonio 1998). Grain rotations that include legumes have been shown to use 12-34% less energy, and pulses use 35-60% less fossil energy compared to grain crops (Jensen et al. 2012). Despite these benefits, there is only one legume variety trial and no research levee in the region (Jacob, Carrasco, and Schwember 2016; Rubiales et al. 2015). Currently, farmers considering legume agriculture are also limited by lack of infrastructure for storage and shipping. Public investment in infrastructure and marketing, however, can lead to increased crop diversity, as demonstrated by increased pea planting following the construction of a publicly funded granary in western Nebraska (Stepanovic et al. 2016). Therefore, we recommend that the USDA allocate funding to sustained research and market development for regionally-adapted legumes as an essential step towards resilient agriculture.

Advantages

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[7] Pulse crops are leguminous crops harvested for their dry seed and tend to have a short growing period.
[8] Forage crops are grown for the edible plant tissue (e.g. leaves and stems, not grain) as animal feed.

- Public investment in crop breeding and market development has potential for a high rate of return (Rubiales et al. 2015).
- Current lack of funding in regionally-adapted legumes means that moderate investments will likely provide substantial gains (Rubiales et al. 2015).
- Increased legume cropping will promote soil health and carbon sequestration.
- Diverse alternatives for a fallow period would increase the impact of FCIP fallow requirement elimination (Option 1).

Disadvantages

- Long-term investment in research, infrastructure, and market development requires resources and political will.
- Mitigation of the rapid and catastrophic consequences of climate change requires action at a pace faster than that of research and market development.

iii. Option 3: Phase out single-crop, yield-based coverage and incentivize whole farm insurance

Most FCIP products insure individual crops based on yield history rather than farm income (Hamilton 2020). The emphasis on yield and penalty[9] for any management that may compromise yield in the short-term, such as cover or continuous cropping, has led farmers to focus on a few high-value, high-yielding crops that rely on high levels of inputs (e.g., fertilizers and herbicides) at the expense of long-term soil health and resiliency (Hamilton 2020). Additionally, the current structure of FCIP may discourage planning for extreme events such as

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[9] Any practice that does not meet the Risk Management Agency's (RMA) description of a "good farming practice" could lose coverage. A good practice is one that "produce[s] the insured crop and allow[s] it to make normal progress toward maturity and produce at least the yield used to determine the production guarantee or amount of insurance [...]" (RMA, USDA 2020 p33-4). (Risk Management Agency 2016).

increased frequency and duration of drought and the number of days above 32°C in a season. A study found insured corn was 67% more prone to extreme heat than uninsured corn (Annan and Schlenker 2015), suggesting that uninsured farms were more proactive in protecting against this common cause of crop loss, for example by planting at a crop density likely to provide a yield even under hot, dry conditions. Rather than insuring single crops based on yield, an alternative is to insure farm revenue, thus removing the emphasis on particular practices or crops while still protecting farms against devastating losses (Hamilton 2020).

The 2014 Farm Bill created such an insurance option called Whole Farm Revenue Protection (WFRP), though less than 2% of growers participate due to reluctance to change and unfamiliarity with the program (Mulik 2017). Incentivizing WFRP while phasing out subsidies for single-crop insurance could promote holistic planning and risk-management while reducing barriers to diversified rotations (Beckie et al. 2019). WFRP also offers an opportunity to develop a definition of a "good farming practice" that includes protection and regeneration of soil health, addresses GHG emissions reduction, and prioritizes or requires crop diversity, as is done in the European Union's Common Agriculture Policy (Meynard et al. 2013).

As the WFRP program has been in place with little adoption, we recommend that the USDA develop specific guidelines for how to transition between programs in collaboration with farmers and relevant member-based organizations. Genuine opportunity to inform policy and incorporate long-term planning into what constitutes a "good farming practice" could promote farmer buy-in with such a transition, enhance adoption of conservation agriculture, and improve the quality and efficacy of FCIP (Lovett 1999). Ultimately, shifting to WFRP while incentivizing conservation agriculture will reduce overall cost of insurance offerings by building farm resiliency (Bryant and O’Connor 2017; Beckie et al. 2019).

Advantages

- WFRP is an existing FCIP option that eliminates many of the institutional drivers towards monoculture and energy-intensive agriculture. Thus, no new programs or funding mechanisms are required to transition away from single-crop policies.
- Collaboration with growers about WFRP policies can encourage adoption of conservation agricultural practices.
- Increasing subsidies for WFRP while gradually reducing subsidies for single-crop insurance would provide a transition period for growers to adopt different agricultural practices while reducing taxpayer risk to rising costs of FCIP.

Disadvantages

- This approach may be met with resistance, as insurance agents with established customers are reluctant to promote WFRP given its reliance on tax records instead of yield history. Insurance companies currently require more documentation for WFRP than single-crop insurance such as proof of markets, receipts for fertilizer and herbicide to satisfy what insurance agents consider “good farming practices”, though such receipts are not requested for other insurance policies. The additional paperwork and research for specialty crops creates an additional bureaucratic burden that impedes adoption by many commodity farmers that could be eliminated. This transition would require collaboration with growers and careful planning to be politically feasible.

4. Synergistic benefits of combined policies

Alone, each policy option is an improvement over the status quo but will not achieve the goal of creating climate-resilient farms. We propose three changes as key elements of a pilot program in keeping with USDA tradition (Hamilton 2020) so they can be ground-tested, modified, and scaled effectively. Together, these changes address bureaucratic obstacles, provide a range of short- and long-term solutions, and promote economic and agricultural sustainability (Fig. 5.1). The policies extend across a spectrum of cost, legislative burden, political capital, and time-scales for implementation and impact, offering a balanced and gradual transition to conservation agriculture. Adopted jointly, these recommendations improve farm resilience to climate change by promoting soil health and crop diversification while reducing GHG emissions. Changes may be met with resistance by individuals and organizations unconvinced of the value of crop diversification or the need to adapt to climate change. A successful transition demands creativity and collaboration. This multifaceted solution is more likely to shift deeply-ingrained practices, however, by creating new opportunities and fewer restrictions on grower decision-making. Furthermore, while the proposed investment in research and market development requires long-term commitments, rates of return can often reach 12-40 to 1 (Malla and Brewin 2019). In combination, these improvements will reduce taxpayer burden to provide farm revenue protection in the face of increasing extreme weather events by improving agricultural resilience.

5. Policy Recommendation

We propose the synergistic combination of changes to FCIP as the most sustainable long-term solution. The USDA can evolve the FCIP to support climate-resilient agriculture by eliminating county-level fallow requirements, funding research and market development for regionally-adapted legumes, and phasing out single-crop, yield-based coverage while incentivizing whole farm insurance. As the rising costs and risks of extreme weather threaten agricultural sustainability, the USDA must act quickly to adjust its far-reaching FCIP program to support practices that reduce fossil energy use and promote soil health and climate resiliency through nature-based solutions.

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CHAPTER 6: CONCLUSION

This work has been a series of small studies assessing the potential for some traditional ideas from nature and our own past to be of value within today's context of agriculture and society. Agriculture has always come at a cost to natural ecosystems – a cost paid in productivity, soil, nutrient retention, biodiversity, water quality, and more. In the past, people have overcome the nagging unsustainability of our collective lifestyle by moving to new places, literally breaking new grounds. As that's become more challenging, extracting nutrients from distant shores and using energy sequestered millennia before our species existed has not only supported civilization, but it led to rapid growth both in our numbers and our level of consumption. The systems we've built often feel so entrenched and powerful, our biggest challenge may be overcoming our own inertia and resistance to reimagining how we might relate to each other and our world in a mutual rather than extractive way, if such a thing is possible for an omnivore.

When I think of how the research and ideas presented here fits within this broader motivation, is there anything I've learned that could carry us forward? I would say it is this: We have to start somewhere. Small changes may not solve any problems, but they can reduce further harm. Incorporating legumes into dryland annual grains seems like it may help rebuild SOM and do so in a region where that is difficult to achieve. Peas are a great choice because they can provide some income (unlike cover crops), and their short growing season means they won't deplete the soil water much compared to longer-lived crops. Even so, many farmers may need support in growing a new crop – everything from infrastructure to regional marketing new crops. Tweaks to USDA funding and the crop insurance program as I proposed in Chapter 5 could incentivize these changes and more and ultimately take a small step towards a system that values soil as much as it does yield.

And what if the study results were wrong? It was an observational study with potential for selection bias in the farms we sampled. The model that affirmed our results might give us some confidence in our findings, but that, too, could be biased by our own thinking. I'd argue the benefits of incorporating legumes and crop diversity, particularly if they extend the time the soil is covered, will contribute to soil health and farm resilience. A more rigorous way to confirm these findings, however, would be to resample these farms five and ten years hence to assess how the system had changed. These types of longer-term, on-farm studies are rare because they do not fit within a typical funding or graduate student cycle, but they could go a long way towards resolving the inherent weakness of comparative, observational studies.

Perennial grains offer a true potential to transform our food system, but with the notable exception of perennial rice, they have a long way to go before they could produce much of our food supply. The question I set out to answer about their ability to sequester SOM had mixed findings. The small observational study found some evidence that Kernza may promote SOC accrual primarily as POM. When I quantified the amount of plant material actually incorporated into the soil, I found that roughly 20% of Kernza root tissue remained after two years, 8% of which was MAOM-C and another 7% as oPOM-C (silt loam texture), suggesting that about 15% of the root tissues may remain "sequestered" in soil. That sound great! Kernza has deep, robust root systems – especially compared to annual crops. Yet when I scaled the likely biomass values to the field-level, the sequestration rates are small. They are at the lowest, low end of accrual rates estimated by other studies. Alfalfa, the legume I hypothesized would be a SOC-storing powerhouse, turned out to disappear within months in the soil, doing some to support Kernza growth but not as much as we might hope (though that might be the gophers' fault), and giving no indication that it could support increased SOC. Of course, this is one study. More research is always needed. I am so curious why legumes sometimes enhance SOC formation and other times have little or no effect. Does it relate to the minerology? Or the particular microbial community active at the time? Is it a quirk of how the research questions are asked and addressed? Could there be a certain type of legume that is actually really good at supporting both N and C accrual where others fail? And what traits make for a good candidate legume? Maybe in exploring these questions we'll revise our understanding of how litter chemistry influences SOM dynamics and the pathways of SOM formation. At least some of the time, it seems you can have too much of a good thing when it comes to litter quality.

Given the potential for intercropped and polyculture perennial grains to alter our relationship to the Earth, we need to understand these dynamics and interactions more deeply. What are the best intercrops? What is the spacing, the timing, the mowing, etc. that optimizes N cycling, fine root growth, efficient microbial metabolism, and SOC accrual? Livestock integration may also be another way to optimize the intercrop system, reduce the need for mowing while potentially supplying readily mineralizable N to the grain crop. The great thing about integrating livestock is that it reduces the need to achieve the maximum yield since the forage has its own value. Farmers I've spoken with emphasized the value of Kernza for its flexibility in this way – being perennial, the timing for management is much more flexible, and cattle can graze sooner and for longer than on corn stover or wheat. What support is needed to encourage more perennial crop livestock integration?

Ultimately the small piece of knowledge this work contributes to the ocean of our understanding is that legumes and perennials both have the potential to positively influence

SOM. That was suspected but not before quantified in the U.S. Central Plains. I have also presented an example of how models could be used to aid observational studies, and how observational studies can test model performance. Finally, I leave you with an idea for systemic change. While small changes matter, systemic changes is how we transform. I believe that each of us, for as long as we’re here, needs to do our part to nudge each other and humanity to be a more mindful and nimble version of ourselves. To that effect, I leave you with a poem I wrote describing a moment when I felt inspired to do so, and it is my intention to pass that breath of hopeful movement onto you.

ADDENDUM

what I wanted to say

His voice fell onto the table softly
the wind that once had sailed vigorously
through buoyant chords
now wafted as a gentle breeze
almost caressing the now-loose strings
rather than plucking them
with the strident definiteness of years past.

He was asked again to hold the microphone closer
so that we could hear
I admired the clarity of his words
Focused, earnest
His audience clearly defined
the 535 elected, innumerate hominids
chosen to sit in Congress
We must speak to these humans
in a language they can hear
Constituents, freedom, security

I smiled
grateful perhaps

charmed
slightly chagrined
to hear someone expose
such bold beliefs
To think, even after all his time
or perhaps because of it,
he still believed
this system could produce anything
of value.

Watching his freckled hands tremble
clutching the microphone
that seemed too heavy
I felt his voice circle those of us in the room
alighting that small spark
the hope that unites us all
that we
somehow
might
make a difference

My eyes responded
by leaking

overwhelmed by the juxtaposition
of fragility and strength

The feeling was familiar
How many times had I hidden
from corporeal realities
by diving into high-minded ideals
sparing myself
if only momentarily
the knowing
the inescapable knowing
that this, too
he, too
I, too
shall pass

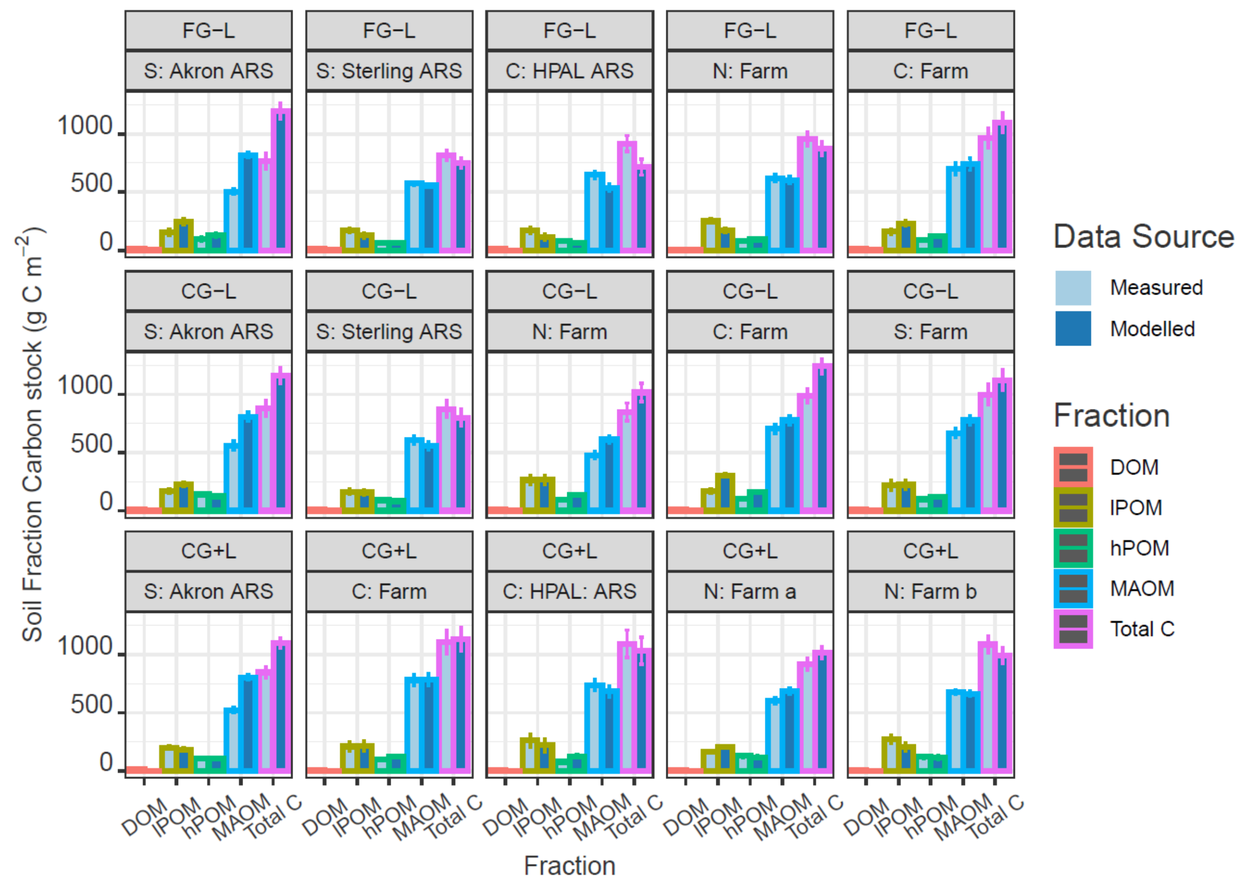
A desire surged within me
to cup those trembling hands
Breathe and affirm the life they still hold
even tenuously
to say
I see you.
I know those fears you wear.

I wear them, too.

Bodies spend each moment fully
committed to life
even as the edges wear off
as strength bleeds away
I felt my Body yearn to share a message
with those hands:
You were born for this.
You were born for this.

APPENDIX

1. Supplementary File for Chapter 2



Supplementary Figure 2.1 Measured and modelled data by soil fraction for each field sampled in 2018. The measured (light color) and modelled (dark color) values for each soil fraction are indicated with the field sites sorted by rotation. S, C, and N indicate geographic regions where S is the Southern region, C the Central region, and N the Northern region where each field was located (Fig. 2.1), and whether a field was a farm or ARS is indicated. Contrasting rotations existed at each ARS (Akron, Sterling, & HPAL) while farms had a single rotation at that site. Colors surrounding the columns vary by soil fraction which are as described in Figure 4. Error bars indicate standard error of the measured values and is used for both data sources; n=6 for Akron ARS and n=8 for all other fields.

Supplementary Table 2.1 Dominant soil types and fertilization for sample fields. Soil type were determined from the USDA Web of Soil Survey (http://websoilsurvey.sc.egov.usda.gov/) where soil types listed were those in the sample area accounting for greater than 10%. Fertilizer was applied for wheat crops with planting in the fall and injected to a depth ~ 15 cm below the soil surface. Fertilization rates for corn were typically 20 lb ac^{-1} higher than for wheat and 20-30 lb ac^{-1} lower for sunflower and millet when fertilized, and pea crops were not fertilized. Rotation categories were as follows: FG-L = fallow-grain without legumes (*wheat fallow)*; CG-L =continuous grain without legumes; CG+L = continuous grain with legumes. Some farms deviated slightly from these exact rotations (see 'Actual Rotation'). Letters represent crops planted in a given year as follows: W=Wheat; C=Corn; M=Millet; P=Pea; Su=Sunflower; So=Sorghum. *n.d.* indicates no data available.

Rotation Category	**Farm or ARS**	**Actual Rotation**	**Soil Type**	**Fertilization for Wheat** (kg ha^{-1})
FG-L	Farm	WFWF	Keith, Duroc, & Alliance Loam	67 N – 34 P
	Farm	WFWF	Rosebud and Alliance Loam	36 N – 0 P
	Akron ARS	WFWF	Platner and Canyon Loam	56-67 N – 0 P
	HPAL ARS	WFWF	Alliance and Kuma Loam	56-67 N – 0 P
	Sterling ARS	WFWF	Rago, Platner, & Weld Loam	45 N – 22 P
CG-L	Farm	WWWW	Rago and Weld silt loam and Platner loam	36 N -- 0 P
	Farm	WMSuC	Keith, Rago, and Kuma silt loam and Ritchfield Loam	77 N – 0 P
	Farm	WMSuC	Tripp very fine sandy loam	56 N – 0 P
	Akron ARS	WCMW	Platner and Canyon Loam	56-67 N – 0 P
	Sterling ARS	WCMSo	Rago, Platner, & Weld Loam	45 N – 22 P
CG+L	Farm	WCPM	Alliance and Rosebud Loam	78 N – 28 P
	Farm	WCMP	Keith and Alliance Loam	*n.d.*
	Farm	WCPW	Rosebud Canyon Loam	36 N -- 0 P
	Akron ARS	WCMP	Platner and Canyon Loam	56-67 N – 0 P
	HPAL ARS	WPMSu	Alliance and Kuma Loam & Sidney-Canyon Complex	56 N – 0 P

Supplementary Table 2.2. Corrected Aikake information criteria (AICc) model selection for relevant covariates for generalized mixed effects linear models. Double asterisk (**) indicate $p < 0.05$; single asterisk (*) denote $p \leq 0.1$. *df* indicates degrees of freedom for the selected model.

		Possible Fixed Covariates for Model Selection					AICc Model Significance				
	Variable of Interest	**%Clay**	**%Sand**	**pH**	**Bulk Density** *(non-stock variables only)*	**MWD** *(0-10 cm only)*	***df***	**Model Weight**	***p* of rotation**	**F Value Rotation**	**Random Variables**
Full Profile (0-100 cm)	**%OC**	x		x	x		9	0.337	0.17	3.39	Regional Grouping; Field, Depth
	%N		x	x	x		8	0.341	**0.13***	**2.48**	
	OC Stock (Mg ha^{-1})	x	x	x			8	0.378	0.42	0.88	
	IC Stock (Mg ha^{-1})	x	x	x			9	0.505	0.35	1.15	
	N Stock (Mg ha^{-1})	x	x				8	0.394	0.34	1.22	
Surface Soil (0-10 cm)	**%OC**		x		x	**x**	8	0.298	0.59	0.52	Regional Grouping; Field
	%N	x	x	**x**	x	x	9	0.307	0.27	1.34	
	2018 Grain Yield (kg ha^{-1})						8	0.358	0.32	1.27	
	4 yr Avg Grain Yield (kg ha^{-1})						7	0.358	**0.04****	**4.86**	
	OC Stock (Mg ha^{-1})	x	x				6	0.366	**0.08***	**3.27**	
	IC Stock (Mg ha^{-1})	x	x	x			9	0.505	0.35	1.15	
	N Stock (Mg ha^{-1})		x				9	0.294	0.28	1.44	
10-30 cm	**OC Stock (Mg ha^{-1})**	x							0.61		
	IC Stock (Mg ha^{-1})	x	x	x					**0.14***	**2.33**	
	N Stock (Mg ha^{-1})	x	x						0.25		
30-50 cm	**OC Stock (Mg ha^{-1})**	x	x						0.77		
	IC Stock (Mg ha^{-1})	x	x	x					**0.10***	**4.90**	
	N Stock (Mg ha^{-1})	x	x						0.29		
50 - 100 cm	**OC Stock (Mg ha^{-1})**	x	x						0.40		
	IC Stock (Mg ha^{-1})	x	x	x					0.72		
	N Stock (Mg ha^{-1})	x	x						0.75		

Supplementary Table 2.3. Literature values used to calculate C inputs to MEMS 1.0 based on yield data (Eqn 2-4).

Crop	**lbs per bushel**	**Planting DOY**	**Harvest DOY**	**No. days with crop**	**Shoot: Root (S:R)**	***S:R Ref***	***S:R Location***	**HI**	***HI Reference***	**HI Location**	**Shoot C** (g C kg^{-1} plant)	**Root C** (g C kg^{-1} plant)	**Shoot CN**	**Root CN**	**Mean CN**	***C & CN Ref***	**C:N Location**
Corn	70	128	268	140	5.6	(Bolinder et al., 2007)	Mead, NE	0.52	(Unkovich et al., 2010, chap. 5)	Australia	463	481	81	35	58	*Shoot:* (Stewart et al., 2015) *Root:*(Redin et al., 2014)	Shoot: Eastern CO; Root: Brazil
Proso millet	50	152	244	92	5.6	*Sterling, CO LTAR; Inferred to be similar to corn*	Sterling, CO	0.41	*Peterson G.A., and Westfall D.G. (1997); M. Schipanski - Sterling, CO LTAR*	Sterling, CO	460	448	45	39	42	*Shoot:* (Redin et al., 2018) *Root:* (Redin et al., 2014)	Shoot & Root: Brazil
Pea	60	91	196	105	5.3	(Gan et al., 2009)	Saskatchewan Canada	0.36	(Unkovich et al., 2010, chap. 5)	Australia	470	309	25	13	19	*Shoot CN:* (Goh and Tutua, 2007)*; Root&Shoot C:* (Redin et al., 2018)	Shoot: New Zealand; Root: Brazil
Winter wheat	60	250	196	311	4.9	(Gan et al., 2009)	Saskatchewa, Canada	0.36	(Gan et al., 2009; Unkovich et al., 2010, chap. 5)	Australia; Canada	444	464	153	42	98	*Shoot:* (Stewart et al., 2015)*; Root:*(Redin et al., 2014)	Shoot: Eastern CO; Root: Brazil
Sunflower	30	152	278	126	5.1	(Redin et al., 2018)	Brazil	0.31	*Peterson et al 1997 (TB97-3)*	Colorado	439	469	149	90	120	*Shoot: Stewart et al., 2015; Root:Redin et al., 2014*	Shoot: Eastern CO; Root: Brazil
Sorghum	50	152	268	116	5.6	*Sterling, CO LTAR; Inferred to be similar to corn*	Sterling, CO	0.58	(Unkovich et al., 2010, chap. 5)	Australia	425	448	82	39	60	*Shoot: Stewart et al., 2015; Root:Redin et al., 2014*	Shoot: Eastern CO; Root: Brazil

2. References

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Supplementary Table 2.4. Rotation effects on soil organic matter C and N fractions (0-10 cm). Analysis of variance (ANOVA) based on linear mixed-effects models with sand as a fixed-effect covariate and site as a random blocking variable. Tukey's Honest Significant Difference (HSD) with Bonferroni correction for multiple comparisons was used for pair-wise comparisons between each continuous rotation and FG-L where ANOVA implicated a meaningful difference between rotations. There were no significant differences between the two continuous rotations. Bolded values indicate $p \leq 0.05$. Differences between continuous rotations and FG-L and mean values with standard error (SE) values displayed in Mg ha^{-1}. Rotations are as described in Table 2.1.

Carbon (Mg C ha^{-1})											
		ANOVA		**CG-L** - FG-L				***CG+L*** - FG-L			
Soil Fraction	***n***	**F**	***p***	***tukey***	***p***	**Diff (mean)**	***SE***	***tukey***	***p***	**Diff** (mean)	***SE***
MAOM	*114*	4.39	***0.02***			0.44 (6.4)	0.3	2.91	***0.01***	0.86 (6.9)	0.3
hPOM	*114*	4.21	***0.02***	2.30	*0.07*	0.17 (1.0)	0.05	2.68	***0.03***	0.20 (1.1)	0.05
lPOM	*114*	1.50	0.20			-0.19 (1.8)	0.2			0.21 (2.2)	0.2
DOM	*114*	3.22	***0.05***			0.02 (0.13)	0.01	2.51	***0.04***	0.05 (0.16)	0.01

Nitrogen (Mg N ha^{-1})											
		ANOVA		**CG-L** - FG-L				***CG+L*** - FG-L			
Soil Fraction	***n***	**F**	***p***	***tukey***	***p***	**Difference (mean)**	***SE***	***tukey***	***p***	**Difference (mean)**	***SE***
MAOM	*114*	3.30	***0.04***			0.04 (0.80)	0.03	2.54	***0.03***	0.08 (0.84)	0.03
hPOM	*114*	2.25	***0.11***			0.02 (0.14)	0.01	2.05	***0.11***	0.02 (0.15)	0.01
lPOM	*114*	1.70	0.20			0.01 (0.13)	0.01	1.79	0.19	0.03 (0.16)	0.01
DOM	*114*	2.05	***0.13***			0.002 (0.012)	0.002	2.01	***0.10***	0.003 (0.014)	0.002

Supplementary Table 2.5. Wilcox sign-rank paired test and goodness of fit (GOF) comparing measured and modeled fraction carbon (C) values for 2018. Negative values indicate that the measured values were greater than modelled estimates, while positive values indicate the opposite; low p-values (**bold**) indicate significant difference between the measured and modeled values (df=14). 95% CI indicates the confidence interval (CI) between which 95% of observations lie. RMSE (root mean squared error) is the square root of the residuals (difference between actual and predicted values) which is an absolute measure of fit. The CI, median, and RMSE values are in Mg C ha^{-1}. Asterisk (*) indicates model fit improvements when the Akron ARS sites were removed from the analysis. Linear regression was performed for each fraction individually as in Fig. 2.6. Fractions are as described in Figure 4.

Fraction	***Wilcox test p-value***	**95% CI**	**Median difference**	**Slope**	**Intercept**	**R^2**	**RMSE**
All Fractions	*0.81*	-0.08 – 0.17	0.02	0.92	0.07	0.96	0.41
Total C	***0.11***	-0.34 – 1.8	0.82	0.11	8.26	0.03	0.98
MAOM	***0.14***	-0.15 – 1.43	0.56	0.07	5.8	0.01	0.85
hPOM	***0.06***	-0.01 – 0.28	0.14	0.36	0.58	0.22	0.17
lPOM	*0.89*	-0.3 – 0.4	0.01	0.10	1.80	0.01	0.42
DOM	***<0.0001***	-0.10 – -0.08	-0.09	1.8	0.07	0.12	0.01
Analysis without the Akron ARS Sites (n=3)							
All Fractions	*0.91*	-0.08 – 0.18	0.02	0.92	0.09	0.97	0.42
Total C*	*0.57*	-0.77 – 1.16	0.22	0.33	6.4	0.32	0.76
MAOM*	*0.68*	-0.34 – 0.60	0.12	0.57	2.7	0.42	0.59
hPOM	***0.05***	-0.02 – 0.34	0.17	0.33	0.58	0.27	0.15
lPOM	0.67	-0.43 – 0.37	-0.06	0.19	1.68	0.05	0.42
DOM	***0.0005***	-0.09 – -0.08	-0.09	1.17	0.08	0.09	0.01