DISSERTATION

# BRIDGING THE GAP BETWEEN REGENERATIVE AGRICULTURE AND THE BIOLOGICAL MECHANISMS CONTROLLING SOIL ORGANIC MATTER DYNAMICS

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# ABSTRACT

# BRIDGING THE GAP BETWEEN REGENERATIVE AGRICULTURE AND THE BIOLOGICAL MECHANISMS CONTROLLING SOIL ORGANIC MATTER DYNAMICS

This dissertation investigates the complex impacts of regenerative agriculture on soil organic matter (SOM) dynamics and soil fauna biodiversity, addressing a broad range of objectives from uncovering global patterns and policy needs to mechanistic understanding. Through global meta-analyses, policy evaluations, field studies, and mechanistic experiments, this research provides a comprehensive understanding of how regenerative practices influence soil health, carbon sequestration, and biodiversity.

Chapter 2 aimed to understand global patterns through a meta-analysis quantifying the effects no-till (NT) and cropping system intensification significantly increase SOM, via impacts on both particulate organic matter (POM) and mineral-associated organic matter (MAOM). The analysis reveals that NT and cropping intensification synergize with integrated crop-livestock (ICL) systems to greatly enhance soil organic carbon (SOC) stocks, highlighting the potential of regenerative practices to mitigate climate change and promote soil health.

Chapter 3 sought to evaluate the impacts of diversified agricultural systems on SOC, soil health, and yield across the United States. The findings indicate that diversified systems consistently show higher levels of SOC, improved soil health, and improved agronomic outcomes. The policy recommendations include increasing funding for soil health practices, supporting longer participation of producers in conservation programs, and tailoring these programs regionally to maximize their effectiveness.

Chapter 4 focused on field-level impacts by examining the effects of varying degrees of regenerative practice adoption on SOM dynamics and soil fauna biodiversity in 22 farms within the Cheney Watershed, of central Kansas. By developing a Regenerative Farming Index (RFI), the study clearly links regenerative practices to increased carbon and nitrogen stocks in both POM and MAOM, and indicates a positive correlation between regenerative practices and soil biodiversity. Path analysis suggests that soil fauna indirectly influence SOM through their role in enhancing regenerative practices.

Chapter 5 aimed to provide a mechanistic understanding of SOM dynamics by exploring the interactions between predatory mites and bacterivorous nematodes. The study highlights how these interactions shape microbial necromass accrual and MAOM formation. The findings underscore the importance of considering the entire soil food web in ecological studies to fully understand SOM formation and stabilization mechanisms.

Overall, this dissertation advances the understanding of SOC dynamics under regenerative agriculture, providing valuable insights for sustainable soil management and climate change mitigation. By integrating global and local scales, it offers a holistic view of how regenerative practices can restore soil health and contribute to more resilient and productive agricultural systems.

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# CHAPTER 1: INTRODUCTION

## 1. BACKGROUND

Soils are among Earth's most vital resources, controlling a diverse array of ecosystem services including the provisioning of food and fiber, decomposition and nutrient cycling, water filtration, and the maintenance of terrestrial biodiversity (Smith et al., 2015). However, agricultural practices, particularly those associated with conventional farming, have significantly altered soil structure and function, often leading to soil degradation, loss of biodiversity, and reduced soil organic matter (Montgomery, 2007). Agriculture uses about one-third of global land, contributes around 15% of global greenhouse gas emissions, and is responsible for an estimated 133 petagrams of soil organic carbon (C) loss, while the need for increased food production continues to rise (Bodirsky et al., 2015; Sanderman et al., 2017). Ensuring that soils remain healthy is crucial for sustaining crop production, despite the challenges posed by climate change and the pressures of extractive agriculture (FAO, 2020). Regenerative agriculture has emerged as a promising solution to these challenges by reducing environmental impacts and enhancing social benefits. Implementing regenerative practices can address the intertwined challenges of soil degradation, biodiversity loss, and climate change while enhancing agricultural productivity and resilience (Rhodes, 2017; Schreefel et al., 2020; Giller et al., 2021).

Soil organic matter (SOM) is critical for soil health, contributing to nutrient availability, water retention, and soil structure (Smith et al., 2015; Baveye et al., 2016). Studying SOM is essential because it not only supports soil biodiversity and plant growth but also acts as a significant C sink, helping to mitigate climate change (Lal, 2004; Schmidt et al., 2011). Improving SOM through regenerative agriculture can harness these benefits, making soils more resilient and productive (Giller et al., 2021; Meena et al., 2024).

Soils support diverse microbial and faunal communities that drive key ecological functions (Bardgett and van der Putten, 2014). These belowground communities are increasingly threatened by land degradation due to agricultural expansion and simplification (Tsiafouli et al., 2015; de Graaff et al., 2019). Managing soil health effectively requires understanding the biological processes driven by soil organisms and their responses to management (Lavelle, 1997; Altieri, 1999; Kibblewhite et al., 2008; Parker, 2010). Soil fauna regulate nutrient fluxes, SOM dynamics, soil structure, and pest suppression (Altieri, 1999; Lavelle et al., 2006, 2020; Barrios, 2007; Brussaard et al., 2007; Smith et al., 2015). Their roles in these processes are vital for regenerative agricultural systems (Doran et al., 2002).

Quantitative assessments of the combined impacts of various regenerative practices on SOM dynamics and soil fauna diversity are limited. Additionally, the mechanisms through which regenerative management affects SOM dynamics and soil fauna are not well understood. This dissertation investigates how regenerative agriculture practices enhance SOM and soil biodiversity, and explores the mechanisms by which soil fauna contribute to SOM formation and stabilization. This research is guided by the two-pathway model of SOM formation, providing insights for policy focused on improving soil health and carbon sequestration through targeted management practices (Cotrufo et al., 2015; Cotrufo and Lavallee, 2022).

## 2. THE PHILOSOPHY OF REGENERATIVE AGRICULTURE

The term “regenerative agriculture” dates back to the 1980s, with pioneers like Wes Jackson and Robert Rodale advocating for the restoration of agricultural resources degraded by conventional practices to achieve sustainability (Jackson, 1980; Rodale, 1983). However, the concept is rooted in ancient agricultural practices, drawing from the deep ecological understanding and traditional knowledge of indigenous peoples. These practices emphasize the reciprocal relationship between humans and the environment, promoting sustainable food production long before modern agricultural methods emerged. This approach offers a tremendous opportunity to restore soil functioning and reduce atmospheric $CO_2$

concentrations (Lal, 2004; Minasny et al., 2017; Zomer et al., 2017). The soil carbon debt, largely due to practices like tillage, bare fallowing, and replacing diverse perennials with annual crops, can be addressed through regenerative practices (Crews and Rumsey, 2017; Sanderman et al., 2017).

Awareness of the need for alternative management practices to maintain or improve productivity while preventing soil degradation has led to the development of management paradigms such as organic farming, agroecology, climate-smart agriculture, and sustainable intensification (Godfray and Garnett, 2014; Lal, 2015; Lampkin et al., 2015; Paustian et al., 2016). These approaches frequently draw on similar practices, including reducing soil tillage intensity, growing cover crops, and integrating perennials and livestock into arable rotations. Such practices are valued for their potential to increase SOM and contribute to climate change mitigation through soil C sequestration. What distinguishes regenerative agriculture is its holistic approach, emphasizing the restoration and enhancement of ecosystems while incorporating social and economic components to improve the overall sustainability of food production (Giller et al., 2021; Bless et al., 2023). Effective policy development is crucial to support the widespread adoption of regenerative practices and achieve meaningful impacts on soil health and climate resilience.

### *2.1 Core principles*

The core principles of regenerative agriculture include minimizing soil disturbance, maintaining continuous vegetation cover, and increasing the quantity and diversity of organic residues returned to the soil. These principles are embodied in various practices:

1. Principle: Minimize Soil Disturbance

Practices include: No-till farming, amendment with organic nutrients (e.g., compost, manure), and organic pest control (e.g., pollinator strips).

Benefits: Enhances SOM stabilization through improved aggregation, soil structure, and reduced erosion (Six et al., 1999; Franzluebbers, 2010; Luo et al., 2010; Ogle et al., 2012; Mondal et al., 2020; Lee et al., 2021).

2. Principle: Maintain Continuous Cover

Practices include: Cover crops, cropping system intensification (eliminating summer fallow, increasing the number of annual crops grown per year), and including perennial crops in rotation.

Benefits: Increases SOM by utilizing otherwise fallow periods, enhancing root carbon inputs (Guo and Gifford, 2002; Ogle et al., 2005; Conant et al., 2017; Husáková et al., 2018; King and Blesh, 2018; Fulton-Smith and Cotrufo, 2019; Sokol et al., 2019; Ledo et al., 2020).

3. Principle: Maximize Biodiversity

Practices include: Integration of livestock and diverse crop rotations.

Benefits: Enhances microbial decomposition efficiency, modifies nitrogen cycling, and increases soil C inputs (Acosta-Martínez et al., 2004; Tracy and Zhang, 2008; Tian et al., 2010; Fultz et al., 2013; McDaniel et al., 2014; Brewer and Gaudin, 2020).

### *2.2 The need for synergy*

While practices like no-till and cover cropping have been extensively studied for their potential to stabilize SOM in the topsoil (Luo et al., 2010; Paul et al., 2019; McClelland et al., 2021), the synergies between multiple regenerative practices and their combined impact on SOM are less understood. Implementing a single practice can be beneficial, but there are often trade-offs, such as yield reduction, that can hinder adoption and long-term effectiveness (Jordon et al., 2022). Adopting a systems approach that addresses multiple regenerative agriculture principles can buffer against negative outcomes and provide additive or even synergistic effects for greater and more rapid soil health improvement (Lemaire et al., 2014; Lehman et al., 2019). Although substantial evidence supports individual regenerative practices, their combined impact on SOM and broader sustainability goals remains underexplored. Therefore, understanding these synergies is crucial for advancing regenerative agriculture and achieving sustainable food production.

## 3. CONCEPTUAL FRAMEWORK FOR STUDYING SOM

This research is largely guided by the two-pathway model of SOM formation (Cotrufo et al., 2015), which describes the formation and stabilization of particulate organic matter (POM) and mineral-associated organic matter (MAOM). POM is formed via the fragmentation of structural plant inputs and is primarily protected by physical occlusion in aggregates, but it receives minimal protection from the soil matrix. Due to its limited protection from decomposition, POM typically has relatively short mean residence times unless decomposition is constrained by physical or physiological limits on microbial activity, leading to POM accumulation in certain environments (von Lützow et al., 2007; Cotrufo and Lavallee, 2022). POM plays a crucial role in providing carbon and nutrients for microbial energy use and plant growth and serves as the primary energy source driving biological functions in the soil (Bu et al., 2015; Bailey et al., 2019; Lavallee et al., 2020). In contrast, MAOM is formed through the sorption of microbial necromass, decomposition products, and soluble plant inputs to soil mineral surfaces (Haddix et al., 2016; Kallenbach et al., 2016; Liang et al., 2019). These mineral bonds make MAOM relatively inaccessible to microbial decomposition, resulting in, on average, longer mean residence times compared to POM (von Lützow et al., 2007; Poeplau et al., 2018) and can be used to assess management impacts on SOM stabilization and sequestration of atmospheric $CO_2$ (Hassink, 1997; Cotrufo et al., 2019). Because MAOM is inherently bound to soil minerals, it has a limited storage capacity depending on the available surface area of minerals; this concept is commonly referred to as 'saturation' (Hassink and Whitmore, 1997; Feng et al., 2013).

Understanding the differential responses of POM and MAOM to regenerative agriculture practices can offer critical insights for restoring soil health and enhancing long-term carbon sequestration. These two SOM fractions have distinct pathways for formation and stabilization. By examining how management practices specifically influence POM and MAOM, we can infer the underlying mechanisms driving these changes. This approach allows us to develop new hypotheses about the processes that contribute to SOM dynamics and devise more effective strategies for soil management.

## 4. SOIL FAUNA AND REGENERATIVE AGRICULTURE

Regenerative agriculture not only enhances SOM but can also promote soil biodiversity, which is crucial for maintaining soil functions and resilience (Bender et al., 2016). Soil fauna, such as earthworms, nematodes, and arthropods, play essential roles in organic matter decomposition and nutrient cycling, contributing significantly to SOM formation and stabilization (Lavelle et al., 2006). While certain regenerative practices are known to enhance soil fauna communities (Rizk and Mikhail, 1999; Fonte et al., 2007; Briones and Schmidt, 2017; de Graaff et al., 2019; Kumar et al., 2020; Kelly et al., 2021; Olayemi et al., 2022), the comprehensive impact of systems-level management involving multiple stacked practices on these communities remains unclear. These soil organisms can, in turn, influence soil health and ecosystem properties, ultimately affecting farm productivity and profitability (Franco et al., 2020; Fonte et al., 2023). Further, understanding the distinct contributions of these organisms to POM and MAOM dynamics can provide insights into the mechanisms driving SOM changes under different management practices. This knowledge is vital for developing effective regenerative agriculture strategies that enhance both SOM and soil biodiversity.

## 5. INTEGRATING GLOBAL AND MECHANISTIC PERSPECTIVES ON REGENERATIVE AGRICULTURE

This dissertation explores the multifaceted impacts of regenerative agriculture on SOM dynamics and soil fauna biodiversity, spanning broad objectives from global patterns and policy needs to mechanistic understanding. Through a combination of a global analysis, policy evaluation, field study, and a mechanistic experiment, it offers a comprehensive insight into SOM dynamics under regenerative agriculture. The research highlights the importance of stacking practices to achieve sustainable food production and informs agricultural policies and management practices that enhance both environmental health and agricultural productivity.

The chapters in this dissertation are interconnected, moving from broad-scale analyses to specific mechanistic studies. Chapter 2 sets the stage with a global meta-analysis, establishing the broad potential of regenerative practices to enhance SOM C. Chapter 3 narrows the focus to the US, evaluating the impact of diversified systems on SOM C, soil health, and yield, providing policy recommendations based on empirical findings. Chapter 4 further narrows the focus, examining the effects of regenerative practices on soil fauna biodiversity and SOM dynamics at the farm level. Finally, Chapter 5 delves into the mechanistic interactions between soil fauna and microbial processes, highlighting the biological underpinnings of SOM stabilization. This dissertation advances the understanding of SOM dynamics under regenerative agriculture, providing valuable insights for sustainable soil management and climate change mitigation. By integrating global and local scales, this dissertation offers a holistic view of how regenerative practices can restore soil health and sequester C, contributing to more resilient and productive agricultural systems.

## REFERENCES

Acosta-Martínez, V., Zobeck, T.M., Allen, V., 2004. Soil microbial, chemical and physical properties in continuous cotton and integrated crop–livestock systems. Soil Science Society of America Journal 68, 1875–1884. doi:10.2136/sssaj2004.1875

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems, in: Paoletti, M.G. (Ed.), Invertebrate Biodiversity as Bioindicators of Sustainable Landscapes. Elsevier, Amsterdam, pp. 19–31. doi:10.1016/B978-0-444-50019-9.50005-4

Bailey, V.L., Pries, C.H., Lajtha, K., 2019. What do we know about soil carbon destabilization? Environmental Research Letters 14, 083004. doi:10.1088/1748-9326/ab2c11

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505–511. doi:10.1038/nature13855

Barrios, E., 2007. Soil biota, ecosystem services and land productivity. Ecological Economics, Special Section - Ecosystem Services and Agriculture 64, 269–285. doi:10.1016/j.ecolecon.2007.03.004

Baveye, P.C., Baveye, J., Gowdy, J., 2016. Soil “Ecosystem” Services and Natural Capital: Critical Appraisal of Research on Uncertain Ground. Frontiers in Environmental Science 4. doi:10.3389/fenvs.2016.00041

Bender, S.F., Wagg, C., van der Heijden, M.G.A., 2016. An Underground Revolution: Biodiversity and Soil Ecological Engineering for Agricultural Sustainability. Trends in Ecology & Evolution 31, 440–452. doi:10.1016/j.tree.2016.02.016

Bless, A., Davila, F., Plant, R., 2023. A genealogy of sustainable agriculture narratives: implications for the transformative potential of regenerative agriculture. Agriculture and Human Values. doi:10.1007/s10460-023-10444-4

Bodirsky, B.L., Rolinski, S., Biewald, A., Weindl, I., Popp, A., Lotze-Campen, H., 2015. Global Food Demand Scenarios for the 21st Century. PLOS ONE 10, e0139201. doi:10.1371/journal.pone.0139201

Brewer, K.M., Gaudin, A.C.M., 2020. Potential of crop-livestock integration to enhance carbon sequestration and agroecosystem functioning in semi-arid croplands. Soil Biology and Biochemistry 149, 107936. doi:10.1016/j.soilbio.2020.107936

Briones, M.J.I., Schmidt, O., 2017. Conventional tillage decreases the abundance and biomass of earthworms and alters their community structure in a global meta-analysis. Global Change Biology 23, 4396–4419. doi:10.1111/gcb.13744

Brussaard, L., de Ruiter, P.C., Brown, G.G., 2007. Soil biodiversity for agricultural sustainability. Agriculture, Ecosystems & Environment, Biodiversity in Agricultural Landscapes: Investing without Losing Interest 121, 233–244. doi:10.1016/j.agee.2006.12.013

Bu, R., Lu, J., Ren, T., Liu, B., Li, X., Cong, R., 2015. Particulate organic matter affects soil nitrogen mineralization under two crop rotation systems. PLoS ONE 10, e0143835. doi:10.1371/journal.pone.0143835

Conant, R.T., Cerri, C.E.P., Osborne, B.B., Paustian, K., 2017. Grassland management impacts on soil carbon stocks: a new synthesis. Ecological Applications 27, 662–668. doi:10.1002/eap.1473

Cotrufo, M.F., Lavallee, J.M., 2022. Chapter One - Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration, in:

Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 1–66. doi:10.1016/bs.agron.2021.11.002

Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. Nature Geoscience 12, 989–994. doi:10.1038/s41561-019-0484-6

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience 8, 776–779. doi:10.1038/ngeo2520

Crews, T.E., Rumsey, B.E., 2017. What agriculture can learn from native ecosystems in building soil organic matter: A review. Sustainability 9, 578. doi:10.3390/su9040578

de Graaff, M.-A., Hornslein, N., Throop, H.L., Kardol, P., van Diepen, L.T.A., 2019. Chapter One - Effects of agricultural intensification on soil biodiversity and implications for ecosystem functioning: A meta-analysis, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 1–44. doi:10.1016/bs.agron.2019.01.001

FAO, 2020. Food and Agriculture Organization of the United Nations: FAO soils portal [WWW Document]. URL http://www.fao.org/soils-portal/soil-degradation-restoration/en/ (accessed 1.23.20).

Feng, W., Plante, A.F., Six, J., 2013. Improving estimates of maximal organic carbon stabilization by fine soil particles. Biogeochemistry 112, 81–93. doi:10.1007/s10533-011-9679-7

Fonte, S.J., Hsieh, M., Mueller, N.D., 2023. Earthworms contribute significantly to global food production. Nature Communications 14, 5713. doi:10.1038/s41467-023-41286-7

Fonte, S.J., Kong, A.Y.Y., van Kessel, C., Hendrix, P.F., Six, J., 2007. Influence of earthworm activity on aggregate-associated carbon and nitrogen dynamics differs with agroecosystem management. Soil Biology and Biochemistry 39, 1014–1022. doi:10.1016/j.soilbio.2006.11.011

Franco, A.L.C., Fonte, S.J., Wall, D.H., 2020. 13 Managing soil biology, in: The Soil-Human Health-Nexus. CRC Press, p. 275.

Franzluebbers, A.J., 2010. Achieving soil organic carbon sequestration with conservation agricultural systems in the southeastern United States. Soil Science Society of America Journal 74, 347–357. doi:10.2136/sssaj2009.0079

Fulton-Smith, S., Cotrufo, M.F., 2019. Pathways of soil organic matter formation from above and belowground inputs in a Sorghum bicolor bioenergy crop. GCB Bioenergy 11, 971–987. doi:10.1111/gcbb.12598

Fultz, L.M., Moore-Kucera, J., Zobeck, T.M., Acosta-Martínez, V., Allen, V.G., 2013. Aggregate Carbon Pools after 13 Years of Integrated Crop-Livestock Management in Semiarid Soils. Soil Science Society of America Journal 77, 1659–1666. doi:10.2136/sssaj2012.0423

Giller, K.E., Hijbeek, R., Andersson, J.A., Sumberg, J., 2021. Regenerative Agriculture: An agronomic perspective. Outlook on Agriculture 50, 13–25. doi:10.1177/0030727021998063

Godfray, H.C.J., Garnett, T., 2014. Food security and sustainable intensification. Philosophical Transactions of the Royal Society B: Biological Sciences 369, 20120273. doi:10.1098/rstb.2012.0273

Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8, 345–360. doi:10.1046/j.1354-1013.2002.00486.x

Haddix, M.L., Paul, E.A., Cotrufo, M.F., 2016. Dual, differential isotope labeling shows the preferential movement of labile plant constituents into mineral-bonded soil organic matter. Global Change Biology 22, 2301–2312. doi:10.1111/gcb.13237

Hassink, J., 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant and Soil 191, 77–87. doi:10.1023/A:1004213929699

Hassink, J., Whitmore, A.P., 1997. A model of the physical protection of organic matter in soils. Soil Science Society of America Journal 61, 131–139. doi:10.2136/sssaj1997.03615995006100010020x

Husáková, I., Weiner, J., Münzbergová, Z., 2018. Species traits and shoot–root biomass allocation in 20 dry-grassland species. Journal of Plant Ecology 11, 273–285. doi:10.1093/jpe/rtw143

Jackson, W., 1980. New Roots for Agriculture. U of Nebraska Press.

Jordon, M.W., Willis, K.J., Bürkner, P.-C., Haddaway, N.R., Smith, P., Petrokofsky, G., 2022. Temperate Regenerative Agriculture practices increase soil carbon but not crop yield—a meta-analysis. Environmental Research Letters 17, 093001. doi:10.1088/1748-9326/ac8609

Kallenbach, C.M., Frey, S.D., Grandy, A.S., 2016. Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. Nature Communications 7, 13630. doi:10.1038/ncomms13630

Kelly, C., Fonte, S., Shrestha, A., Daane, K., Mitchell, J., 2021. Winter cover crops and no-till promote soil macrofauna communities in irrigated, Mediterranean cropland in California, USA. Applied Soil Ecology 166. doi:10.1016/j.apsoil.2021.104068

Kibblewhite, M.G., Ritz, K., Swift, M.J., 2008. Soil health in agricultural systems. Philosophical Transactions of the Royal Society B: Biological Sciences 363, 685–701. doi:10.1098/rstb.2007.2178

King, A.E., Blesh, J., 2018. Crop rotations for increased soil carbon: perenniality as a guiding principle. Ecological Applications 28, 249–261. doi:10.1002/eap.1648

Kumar, N.G., Ammagarahalli, B., Gopalkrishna, H.R., 2020. Soil Fauna and Sustainable Agriculture, in: Chakravarthy, A.K. (Ed.), Innovative Pest Management Approaches for the 21st Century: Harnessing Automated Unmanned Technologies. Springer, Singapore, pp. 211–226. doi:10.1007/978-981-15-0794-6_11

Lal, R., 2015. Sequestering carbon and increasing productivity by conservation agriculture. Journal of Soil and Water Conservation 70, 55A-62A. doi:10.2489/jswc.70.3.55A

Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304, 1623–1627. doi:10.1126/science.1097396

Lampkin, N., Pearce, B., Leake, A., Creissen, H., Gerrard, C.L., Gerling, R., Lloyd, S., Padel, S., Smith, J., Smith, L., Vieweger, A., Wolfe, M., 2015. The Role of Agroecology in Sustainable Intensification (Report). Organic Research Centre and Game & Wildlife Conservation Trust, Newbury and Fordingbridge.

Lavallee, J.M., Soong, J.L., Cotrufo, M.F., 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. Global Change Biology 26, 261–273. doi:10.1111/gcb.14859

Lavelle, P., 1997. Faunal activities and soil processes: Adaptive strategies that determine ecosystem function, in: Begon, M., Fitter, A.H. (Eds.), Advances in Ecological Research. Academic Press, pp. 93–132. doi:10.1016/S0065-2504(08)60007-0

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P., Rossi, J.-P., 2006. Soil invertebrates and ecosystem services. European Journal of Soil Biology, ICSZ 42, S3–S15. doi:10.1016/j.ejsobi.2006.10.002

Lavelle, P., Spain, A., Fonte, S., Bedano, J.C., Blanchart, E., Galindo, V., Grimaldi, M., Jimenez, J.J., Velasquez, E., Zangerlé, A., 2020. Soil aggregation, ecosystem engineers and the C cycle. Acta Oecologica 105, 103561. doi:10.1016/j.actao.2020.103561

Ledo, A., Smith, P., Zerihun, A., Whitaker, J., Vicente-Vicente, J.L., Qin, Z., McNamara, N.P., Zinn, Y.L., Llorente, M., Liebig, M., Kuhnert, M., Dondini, M., Don, A., Diaz-Pines, E., Datta, A., Bakka, H., Aguilera, E., Hillier, J., 2020. Changes in soil organic carbon under perennial crops. Global Change Biology 26, 4158–4168. doi:10.1111/gcb.15120

Lee, S., Chu, M.L., Guzman, J.A., Botero-Acosta, A., 2021. A comprehensive modeling framework to evaluate soil erosion by water and tillage. Journal of Environmental Management 279, 111631. doi:10.1016/j.jenvman.2020.111631

Lehman, R.M., Osborne, S.L., McGraw, K., 2019. Stacking agricultural management tactics to promote improvements in soil structure and microbial activities. Agronomy 9, 539. doi:10.3390/agronomy9090539

Lemaire, G., Franzluebbers, A., Carvalho, P.C. de F., Dedieu, B., 2014. Integrated crop–livestock systems: Strategies to achieve synergy between agricultural production and environmental quality. Agriculture, Ecosystems & Environment, Integrated Crop-Livestock System Impacts on Environmental Processes 190, 4–8. doi:10.1016/j.agee.2013.08.009

Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Global Change Biology 25, 3578–3590. doi:10.1111/gcb.14781

Luo, Z., Wang, E., Sun, O.J., 2010. Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. Agriculture, Ecosystems & Environment 139, 224–231. doi:10.1016/j.agee.2010.08.006

McClelland, S.C., Paustian, K., Schipanski, M.E., 2021. Management of cover crops in temperate climates influences soil organic carbon stocks: a meta-analysis. Ecological Applications 31, e02278. doi:10.1002/eap.2278

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecological Applications 24, 560–570. doi:10.1890/13-0616.1

Meena, R.S., Singh, A.K., Jatav, S.S., Rai, S., Pradhan, G., Kumar, S., Mina, K.K., Jhariya, M.K., 2024. Chapter 10 - Significance of soil organic carbon for regenerative agriculture and ecosystem services, in: Singh, K., Ribeiro, M.C., Calicioglu, Ö. (Eds.), Biodiversity and Bioeconomy. Elsevier, pp. 217–240. doi:10.1016/B978-0-323-95482-2.00010-9

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.-S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.-C., Vågen, T.-G., van Wesemael, B., Winowiecki, L., 2017. Soil carbon 4 per mille. Geoderma 292, 59–86. doi:10.1016/j.geoderma.2017.01.002

Mondal, S., Chakraborty, D., Bandyopadhyay, K., Aggarwal, P., Rana, D.S., 2020. A global analysis of the impact of zero-tillage on soil physical condition, organic carbon content, and plant root response. Land Degradation & Development 31, 557–567. doi:10.1002/ldr.3470

Montgomery, D.R., 2007. Dirt: The Erosion of Civilizations. University of California Press.

Ogle, S.M., Breidt, F.J., Paustian, K., 2005. Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. Biogeochemistry 72, 87–121. doi:10.1007/s10533-004-0360-2

Ogle, S.M., Swan, A., Paustian, K., 2012. No-till management impacts on crop productivity, carbon input and soil carbon sequestration. Agriculture, Ecosystems & Environment 149, 37–49. doi:10.1016/j.agee.2011.12.010

Olayemi, O., Schneekloth, J., Wallenstein, M., Trivedi, P., Calderon, F., Corwin, J., Fonte, S., 2022. Soil macrofauna and microbial communities respond in similar ways to management drivers in an irrigated maize system of Colorado (USA). Applied Soil Ecology 78. doi:10.1016/j.apsoil.2022.104562

Parker, S.S., 2010. Buried treasure: soil biodiversity and conservation. Biodiversity and Conservation 19, 3743–3756. doi:10.1007/s10531-010-9924-8

Paul, E.A., Elliott, E.T., Paustian, K., Cole, C.V. (Eds.), 2019. Soil Organic Matter in Temperate Agroecosystems: Long-Term Experiments in North America. CRC Press, Boca Raton. doi:10.1201/9780367811693

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G.P., Smith, P., 2016. Climate-smart soils. Nature 532, 49–57. doi:10.1038/nature17174

Poeplau, C., Don, A., Six, J., Kaiser, M., Benbi, D., Chenu, C., Cotrufo, M.F., Derrien, D., Gioacchini, P., Grand, S., Gregorich, E., Griepentrog, M., Gunina, A., Haddix, M., Kuzyakov, Y., Kühnel, A., Macdonald, L.M., Soong, J., Trigalet, S., Vermeire, M.-L., Rovira, P., van Wesemael, B., Wiesmeier, M., Yeasmin, S., Yevdokimov, I., Nieder, R., 2018. Isolating organic carbon fractions with varying turnover rates in temperate agricultural soils – A comprehensive method comparison. Soil Biology and Biochemistry 125, 10–26. doi:10.1016/j.soilbio.2018.06.025

Rhodes, C.J., 2017. The Imperative for Regenerative Agriculture. Science Progress 100, 80–129. doi:10.3184/003685017X14876775256165

Rizk, M.A., Mikhail, W.Z.A., 1999. Impact of no-tillage agriculture on soil fauna diversity. Zoology in the Middle East 18, 113–120. doi:10.1080/09397140.1999.10637787

Rodale, R., 1983. Breaking New Ground: The Search for a Sustainable Agriculture. Futurist 17, 15–20.

Sanderman, J., Hengl, T., Fiske, G.J., 2017. Soil carbon debt of 12,000 years of human land use. Proceedings of the National Academy of Sciences 114, 9575–9580. doi:10.1073/pnas.1706103114

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56. doi:10.1038/nature10386

Schreefel, L., Schulte, R.P.O., de Boer, I.J.M., Schrijver, A.P., van Zanten, H.H.E., 2020. Regenerative agriculture – the soil is the base. Global Food Security 26, 100404. doi:10.1016/j.gfs.2020.100404

Six, J., Elliott, E.T., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Science Society of America Journal 63, 1350–1358. doi:10.2136/sssaj1999.6351350x

Smith, P., Cotrufo, M.F., Rumpel, C., Paustian, K., Kuikman, P.J., Elliott, J.A., McDowell, R., Griffiths, R.I., Asakawa, S., Bustamante, M., House, J.I., Sobocká, J., Harper, R., Pan, G., West, P.C., Gerber, J.S., Clark, J.M., Adhya, T., Scholes, R.J., Scholes, M.C., 2015. Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils. SOIL 1, 665–685. doi:https://doi.org/10.5194/soil-1-665-2015

Sokol, N.W., Kuebbing, S.E., Karlsen-Ayala, E., Bradford, M.A., 2019. Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon. The New Phytologist 221, 233–246. doi:10.1111/nph.15361

Tian, L., Dell, E., Shi, W., 2010. Chemical composition of dissolved organic matter in agroecosystems: Correlations with soil enzyme activity and carbon and nitrogen mineralization. Applied Soil Ecology 46, 426–435. doi:10.1016/j.apsoil.2010.09.007

Tracy, B.F., Zhang, Y., 2008. Soil compaction, corn yield response, and soil nutrient pool dynamics within an integrated crop-livestock system in Illinois. Crop Science 48, 1211–1218. doi:10.2135/cropsci2007.07.0390

Tsiafouli, M.A., Thébault, E., Sgardelis, S.P., de Ruiter, P.C., van der Putten, W.H., Birkhofer, K., Hemerik, L., de Vries, F.T., Bardgett, R.D., Brady, M.V., Bjornlund, L., Jørgensen, H.B., Christensen, S., Hertefeldt, T.D., Hotes, S., Gera Hol, W. h., Frouz, J., Liiri, M., Mortimer, S.R., Setälä, H., Tzanopoulos, J., Uteseny, K., Pižl, V., Stary, J., Wolters, V., Hedlund, K., 2015. Intensive agriculture reduces soil biodiversity across Europe. Global Change Biology 21, 973–985. doi:10.1111/gcb.12752

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biology and Biochemistry 39, 2183–2207. doi:10.1016/j.soilbio.2007.03.007

Zomer, R.J., Bossio, D.A., Sommer, R., Verchot, L.V., 2017. Global sequestration potential of increased organic carbon in cropland soils. Scientific Reports 7, 15554. doi:10.1038/s41598-017-15794-8

# CHAPTER 2: RESTORING PARTICULATE AND MINERAL-ASSOCIATED ORGANIC CARBON THROUGH REGENERATIVE AGRICULTURE[1]

## 1. INTRODUCTION

Since the development of agriculture, an estimated 133 petagrams of soil organic carbon (SOC) have been lost from soils (Sanderman et al., 2017). In addition to contributions to climate change, this SOC loss has imperiled soil fertility and global food security (Paul, 2016; Jayaraman et al., 2021), as SOC stocks are positively related to both yield and yield stability (Pan et al., 2009; Oldfield et al., 2019). 'Re-paying' this SOC deficit presents a tremendous opportunity to restore soil functioning and reduce atmospheric $CO_2$ concentrations (Lal, 2004; Minasny et al., 2017; Zomer et al., 2017). As much of this SOC debt is due to select practices of tillage, bare fallowing, and replacement of diverse perennials with annual crops (Crews and Rumsey, 2017), SOC can be regenerated with climate-smart management (Paustian et al., 2016), also termed regenerative management (Jordon et al., 2022). Regenerative agriculture is an approach to farming that aims to improve not only the environmental but also the social and economic aspects of sustainable food production (Schreefel et al., 2020).

Principles of regenerative agricultural leverage ecological understanding to build SOC and thereby regenerate soil health (Ogle et al., 2005; Paul et al., 2019). These principles include: (1) minimizing soil disturbance; (2) maintaining continuous vegetation cover; and (3) increasing quantity and diversity of organic residues returned to the soil (Crews and Rumsey, 2017). Practices that address one or more of these principles include reduced tillage/no-till (principle 1), cropping system intensification (principles 2 and 3), and the integration of livestock into crop production systems (principle 3). While reduced tillage and no-till have received extensive attention as a category of practices that can promote the stabilization of SOC in the topsoil through enhanced aggregation, improved soil structure, and

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reduced erosion (Six et al., 1999; Franzluebbers, 2010; Luo et al., 2010; Ogle et al., 2012; Mondal et al., 2020; Lee et al., 2021), cropping system intensification and livestock integration are more fluid concepts.

We define cropping system intensification to comprise of four main strategies: i) eliminating summer fallow in monocrop systems, ii) increasing the number of annual crops grown per year, iii) planting cover crops, and iv) including perennial crops in rotation. All of these strategies aim to increase SOC by capitalizing on niches in time that would otherwise be left fallow and unproductive (King and Blesh, 2018). Increasing the number of annual crops grown per year increases SOC directly through greater C inputs and indirectly through increased microbial biomass and soil aggregation (Rosenzweig et al., 2018). Similarly, cover cropping can increase SOC by enhancing the quantity and diversity of carbon (C) inputs to soil (McDaniel et al., 2014; Poeplau and Don, 2015; Abdalla et al., 2019; McClelland et al., 2021). Intensification with perennial crops can greatly increase SOC (Guo and Gifford, 2002; Ogle et al., 2005; Conant et al., 2017; Ledo et al., 2020). Perennials produce larger and deeper roots than annual crops (Husáková et al., 2018), a key factor for SOC accrual as root C inputs are preferentially retained in soil compared to aboveground C inputs (Fulton-Smith and Cotrufo, 2019; Sokol et al., 2019).

While a single regenerative practice can build SOC when used in isolation, the potential for synergies between multiple practices is only hinted at in current work. For example, in a dryland system, planting cover crops is more effective at supporting SOC formation when used in combination with no-till management (McClelland et al., 2021). As a regenerative practice that requires multiple strategies to be simultaneously deployed, integrating livestock into cropping systems has shown promise as a means to increase SOC stocks (Lemaire et al., 2014). Crop-livestock integration (ICL) impacts multiple SOC stabilization pathways by shifting C deposition belowground, modifying N stocks and cycling, and increasing the efficiency of microbial decomposition (Acosta-Martínez et al., 2004; Tracy and Zhang, 2008; Tian et al., 2010; Fultz et al., 2013; Brewer and Gaudin, 2020). Despite the likelihood for regenerative practices in ICL and in other systems to increase SOC when adopted in a ‘stacked’ fashion, large-scale studies to evaluate regenerative practice synergies on SOC are lacking.

Although evidence that regenerative agricultural practices can increase SOC stocks is unequivocal, system responses are context specific and accurate prediction of SOC responses to improved management at the field scale remain arduous (Mondal et al., 2020). We posit that these system-specific responses arise in part because SOC is not a uniform substance. Separating it into particulate (POC) and mineral-associated (MAOC), its two most contrasting forms, can facilitate understanding and prediction of broad-scale SOC dynamics to provide recommendations to managers and policymakers (Lavallee et al., 2020; Just et al., 2021). These two fractions can be separated by size and/or density (Leuthold et al., 2022) and show consistent differences in turnover times (Poeplau et al., 2018). POC, widely considered a key indicator of soil fertility, cycles faster than MAOC and therefore is more vulnerable to disturbance (Lavallee et al., 2020). Conversely, MAOC can be used to assess the capacity for regenerative agriculture to promote SOC sequestration and mitigate atmospheric $CO_2$ (Hassink, 1997; Cotrufo et al., 2019). Indeed, the millennia of SOC loss from agricultural soils was preferentially from the POC fraction, and a low proportion of POC characterize agricultural relative to unmanaged soils (Lugato et al., 2021). However, until now, the extent to which regenerative agriculture can restore soil health or sequester carbon long-term by promoting POC and MAOC formation has not been synthesized at the global scale.

With recent emphasis on regenerative agriculture to regenerate soil fertility and mitigate climate change, it is crucial that we understand how these two distinct pools of SOC (POC and MAOC) respond to management. We therefore conducted the first global systematic meta-analysis of 1) the overall response of POC and MAOC to no-till management and cropping system intensification across the soil profile; 2) the impact of agronomic moderators of no-till and cropping intensification on POC and MAOC; and 3) the potential synergy of multiple regenerative interventions to build POC and MAOC in integrated crop-livestock systems. We also explored the effects of climate and soil variation on the response of POC and MAOC to management. We hypothesized, broadly, that due to assorted pathways for regenerative management to augment total SOC, that regenerative practices would differently affect POC and MAOC.

## 2. RESULTS

### *2.1 Distribution and relative importance of stock change for POC and MAOC*

Overall, 118 studies comprising 157 experiments were analyzed in this study (Appendix) In most soils, POC made up a small proportion (~18%) of total SOC stocks (Mg C ha$^{-1}$) compared to MAOC (Fig. 2.1A). Consequently, a large relative change in the POC concentration (g C kg soil$^{-1}$) resulted in only a minor absolute change in SOC stocks (Fig. 2.1B). For instance, a 50% increase in POC concentration was associated with an average change of only 2.3 Mg C ha$^{-1}$, whereas the same percent increase in MAOC and SOC pools was associated with an average stock change of 8.6 Mg C ha$^{-1}$ and 13.9 Mg C ha$^{-1}$, respectively.

We focus on meta-analytic comparisons of relative change in C concentrations, rather than absolute changes in C stock, due to paucity of bulk density data available and high variation in soil sampling depth increments.

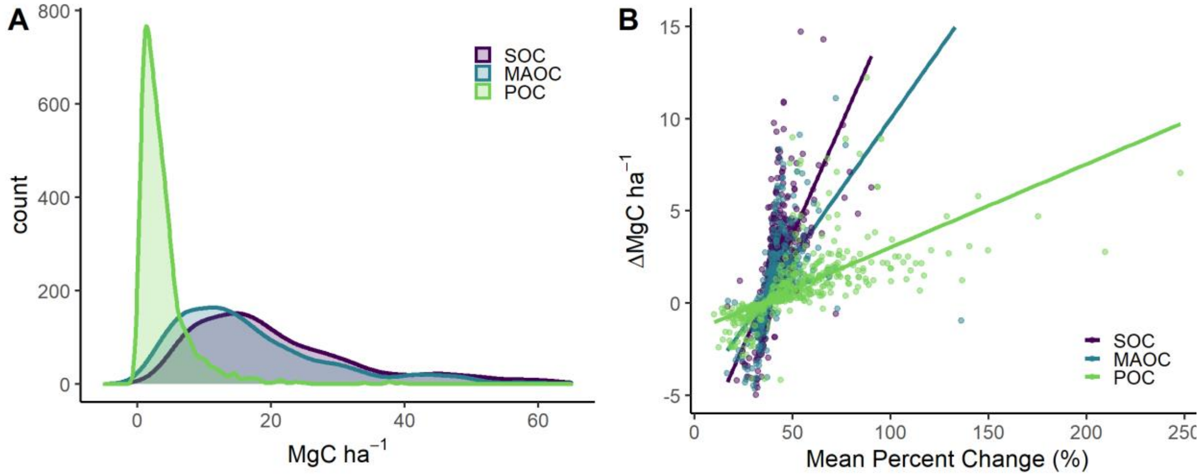

**Figure 2.1. A**, Density plot of carbon (C) stocks (MgC ha$^{-1}$) in total soil organic carbon (SOC), mineral-associated organic carbon (MAOC), and particulate organic carbon (POC). **B**, Plot of linear relationship between Mean Percent Change of C concentrations (gC kg soil$^{-1}$) and absolute response of C stocks (ΔMgC ha$^{-1}$) for SOC, MAOC, and POC.

## *2.2 No-till and cropping system intensification build POC and MAOC in topsoil*

To compare the impact of regenerative management across the soil profile we delineated between topsoil and subsoil at 20 cm, as this was the average tillage depth across studies. Regenerative management significantly increased SOC content in the topsoil but did not significantly affect SOC fractions in subsoil, which had much fewer observations (Fig. 2.2). Compared to conventional tillage (CT), no-till (NT) increased topsoil SOC by 11.3%, with an 8.5% increase in MAOC and a 19.7% increase in POC (Fig. 2.2A). Cropping system intensification as defined above increased SOC by 12.4%, with a 7.1% increase in MAOC and a substantial 33.3% increase in POC (Fig. 2.2A).There was a tendency for a large positive response of POC to cropping intensification in subsoil (+22.5%, Fig. 2.2B), but with substantial variation around the mean (CI: -2.76 – 54.5%), indicating the influence of other moderator effects on POC response.

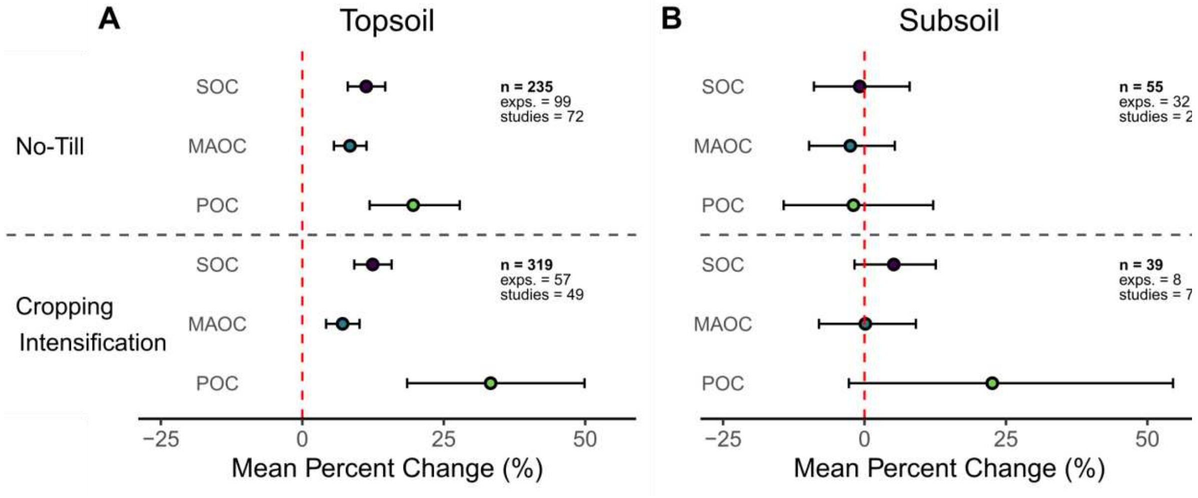

**Figure 2.2. Overall response of carbon concentrations** (g C kg soil$^{-1}$) in total soil organic carbon (SOC), mineral-associated organic carbon (MAOC), and particulate organic carbon (POC) to regenerative agricultural management in topsoil (**A**, 0-20 cm) and subsoil (**B**, 20-100 cm). No-Till systems are compared to conventional tillage systems. Cropping Intensification includes i) eliminating summer fallow in monocrop systems, ii) increasing the number of annual crops grown per year, iii) planting cover crops, and iv) incorporating perennial crops in rotation. Effect sizes were considered significant if 95% confidence interval did not overlap zero.

### *2.3 Effects of no-till on SOC fractions vary with experimental duration, tillage frequency, and cropping system intensity*

Since the response of SOC fractions to NT are only significant in topsoil, we analyzed agronomic practices as moderators effecting the SOC, MAOC, and POC response to NT for topsoil only (Fig. 2.3, Supplementary Table 2.1). We found that NT significantly increased SOC, MAOC, and POC only in experiments lasting greater than 6 years. Tillage frequency moderated the effect of NT on MAOC (Fig. 2.3B), but not on POC (Fig. 2.3C) resulting in a slight trend towards significance in SOC (Fig. 2.3A). Further, MAOC concentrations under NT were 14.2% higher than CT systems with multiple tillage disturbances per year and only 5.0% higher than CT systems with only one tillage disturbance per year (Fig. 2.3B).

Cropping intensity of the rotation moderated the effect of NT on SOC (Fig. 2.3A), MAOC (Fig. 2.3B), and POC (Fig. 2.3C). Compared to CT cropping systems, total SOC increases in NT systems with only one cash crop per year (+13.8%) were significantly higher than SOC increases in systems including multiple cash crops in a year (+6.1%, $p = 0.006$) and systems with cover crops (+6.8%, $p = 0.0001$). For MAOC, NT had the greatest effect in systems with multiple crops per year (+13.3%) compared to systems with cover crops (+7.3%, $p = 0.003$) and systems with one cash crop (+8.1%, $p = 0.10$). The effect of NT on POC was only significantly different than zero in systems with a single cash crop (+32.7%). In systems with multiple cash crops per year and systems with cover crops, NT did not increase POC compared to CT.

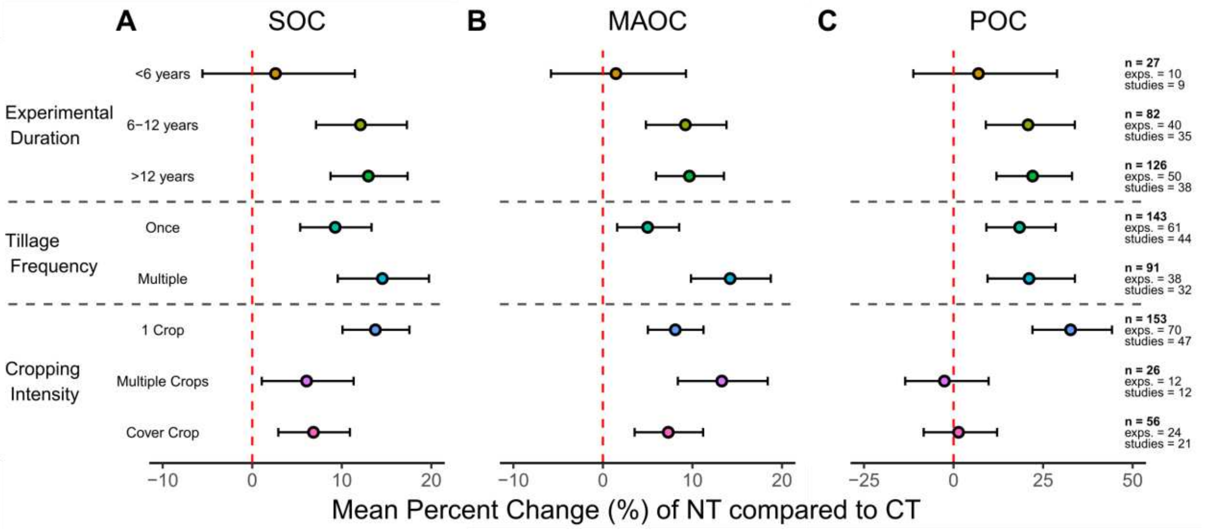

**Figure 2.3. Effect of no-till (NT) compared to conventional tillage (CT)** on topsoil (0-20 cm) carbon concentrations (g C kg soil$^{-1}$) in total soil organic carbon (**A**; SOC), mineral-associated organic carbon (**B**; MAOC), and particulate organic carbon (**C**; POC), as moderated by experimental duration, tillage frequency, and cropping intensity. Cropping rotation, residue management, fertilization regime, and environmental variables held constant. Tillage frequency and cropping intensity on per year basis. Effect sizes were considered significant if 95% confidence interval did not overlap zero. The number of observations (n), experiments (exps.), and studies analyzed for each moderator variable is displayed on the far right of figure.

### *2.4 Effects of cropping system intensification on SOC fractions vary with experimental duration, type of intensification, and tillage management*

Intensification increased C in soil fractions only in experiments lasting greater than 6 years (Fig. 2.4). In experiments that lasted longer than 12 years, intensification increased total SOC by 17.6%, MAOC by 10.8% and POC by 44.0%. These were significantly higher than increases in experiments 6 – 12 years for SOC (+12.3%, $p = 0.012$) and MAOC (+6.5%, $p = 0.039$).

SOC increases were greatest when perennial cropping systems were compared to annual cropping systems (+16.2%), significantly higher than the effect of multiple crop intensification ($p < 0.001$) and cover crop intensification ($p = 0.024$). The response of POC to intensification was greatest when one-crop systems were compared to crop-fallow systems (+57.5%). This effect was significantly higher than the effect of multiple crop ($p$ <0.0001), cover crop ($p$ < 0.0001), and perennial intensification ($p$ = 0.0001) on POC. Further, the effect of cover crops and perennial intensification on SOC and POC were significantly higher than the effect of multiple crops ($p$ < 0.0001). In contrast to SOC and POC, MAOC increases were greatest when comparing systems with multiple crops per year to one-crop systems (+8.9%).

Increasing cropping diversity did not modify the positive effect of intensification on SOC but reduced it for MAOC and POC; with polyculture surprisingly showing the least benefit (Fig. 2.4). The response of SOC, MAOC, and POC to intensification was significantly higher in CT systems compared to NT systems (Fig. 2.4).

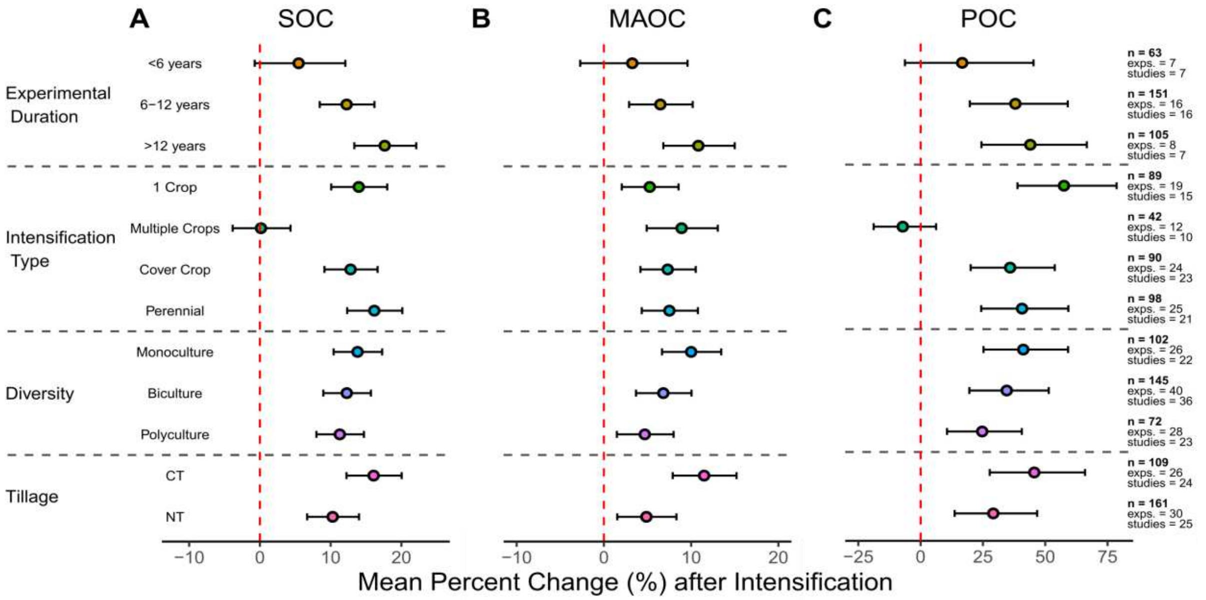

**Figure 2.4. Effect of cropping system intensification** on topsoil (0-20cm) carbon concentrations (g C kg soil$^{-1}$) in total soil organic carbon (**A**; SOC), mineral-associated organic carbon (**B**; MAOC), and particulate organic carbon (**C**; POC) as moderated by experimental duration, type of cropping intensity, rotation diversity, and tillage management. Tillage management, residue management, fertilization regime, and environmental variables were held constant. 'Intensification Type' signifies type of intensification (i.e., '1 crop' = intensification of crop-fallow to rotation of 1 annual cash crop per year, 'Multiple Crops' = intensification of 1 cash crop per year to multiple cash crops per year, 'Cover Crop' = intensification of annual cash crop system to annual rotation + cover crop, 'Perennial' = intensification of annual cropping system to system with a perennial crop included in rotation). Effect sizes were considered significant if 95% confidence interval did not overlap zero. The number of observations (n), experiments (exps.), and studies analyzed for each moderator variable is displayed on the far right of figure.

### *2.5 Potential synergies within integrated crop-livestock systems*

We synthesized the effects of integrating livestock (ICL) in combination with NT and cropping system intensification on SOC, MAOC, and POC in topsoil (Fig. 2.5). Integrating livestock into annual cropping systems had a tendency to increase SOC (Fig. 2.5A) and POC (Fig. 2.5C), but not MAOC (Fig. 2.5B).

Cropping systems that combined livestock integration with NT practices increased total SOC by 29.9% compared to CT systems without livestock, more than double the effect of NT alone (+11.3%). This SOC increase occurred primarily through increases in POC (+38.1%), suggesting an additive effect of combining ICL (+17.0%, ns) with NT (+19.6%). There was a tendency for increased MAOC under NT + ICL (+9.1%) compared to CT without ICL, however, the response was not significantly different from zero.

When livestock integration was used in conjunction with cropping system intensification, C increases were greater than the sum of individual practices. Combing ICL with cover crops has the potential to significantly increase SOC, MAOC, and POC (Fig. 2.5). The 33.1% increase in MAOC suggests a potential synergy between ICL (+1.8%, ns) and cover crops (+7.3%) as it greatly exceeded the additive increase of individual practices. However, the 26.3% increase in POC with ICL + cover crops are lower than POC increases with cover crops alone (+35.9%). The differential responses of POC and MAOC under ICL + cover crops resulted in a 14.6% increase in SOC which was comparable to SOC increases under cover crops alone (+12.8%). Similarly, ICL in combination with perennials increased MAOC by 53.6% showing signs of synergetic effects far exceeding the effects of ICL (+1.8%, ns) and perennial intensification alone (+7.5%). This synergy was not observed for POC or SOC (Fig. 2.5).

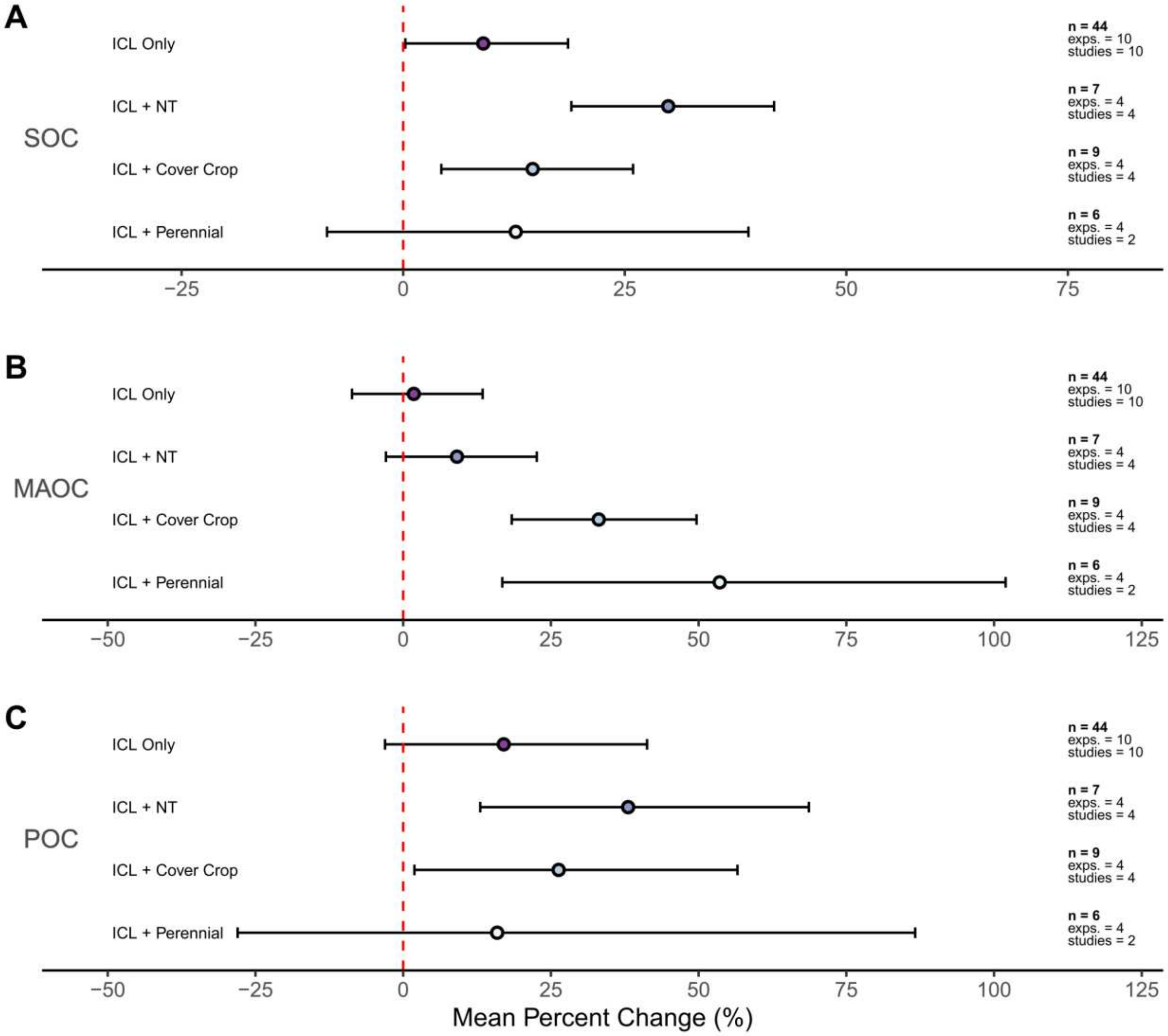

**Figure 2.5. Potential synergistic effects between integrated-crop livestock with no-till and cover crop intensification** on topsoil (0-20cm) carbon concentrations (g C kg soil$^{-1}$) in total soil organic carbon (**A**; SOC), mineral-associated organic carbon (**B**; MAOC), and particulate organic carbon (**C**; POC). ‘ICL Only’ compares ICL systems to annual cropping systems without livestock. ‘ICL + NT’ compares ICL systems that are no-till (NT) to annual systems without livestock that are conventionally tilled. ‘ICL + Cover Crop’ compares ICL systems that have been intensified using cover crops to less intense systems without livestock. ‘ICL + Perennial’ compares ICL systems that have been intensified using perennial grass to annual systems without livestock. Residue management, fertilization regime, and environmental variables were held constant. Effect sizes were considered significant if 95% confidence interval did not overlap zero. The number of observations (n), experiments (exps.), and studies analyzed for each moderator variable is displayed on the far right of figure.

### 2.6 *Regenerative practices effective across a range of soil and climate conditions*

Our synthesis included soils with a wide range of sand content (1.0–89%), soil pH (4.2–8.6), mean annual temperature (MAT; −2.8–30.1 °C), mean annual precipitation (MAP; 302–3100 mm yr$^{-1}$), and potential evapotranspiration (PET; 787–2145 mm yr$^{-1}$). MAT was the only significant moderator of the effect of no-till on SOC, MAOC, and POC (Supplementary Table 2.1). For every 10°C of MAT, the effect of no-till was increased by 8.3% for SOC, by 6.3% for MAOC, and by 14.2% for POC (Fig. 2.6A). For cropping system intensification, the only significant environmental moderator was sand content for SOC and POC, but not MAOC (Supplementary Table 2.2). For every 10% increase in sand content, the effect of intensification was increased by 3.7% for SOC and by 10.6% for POC (Fig. 2.6B).

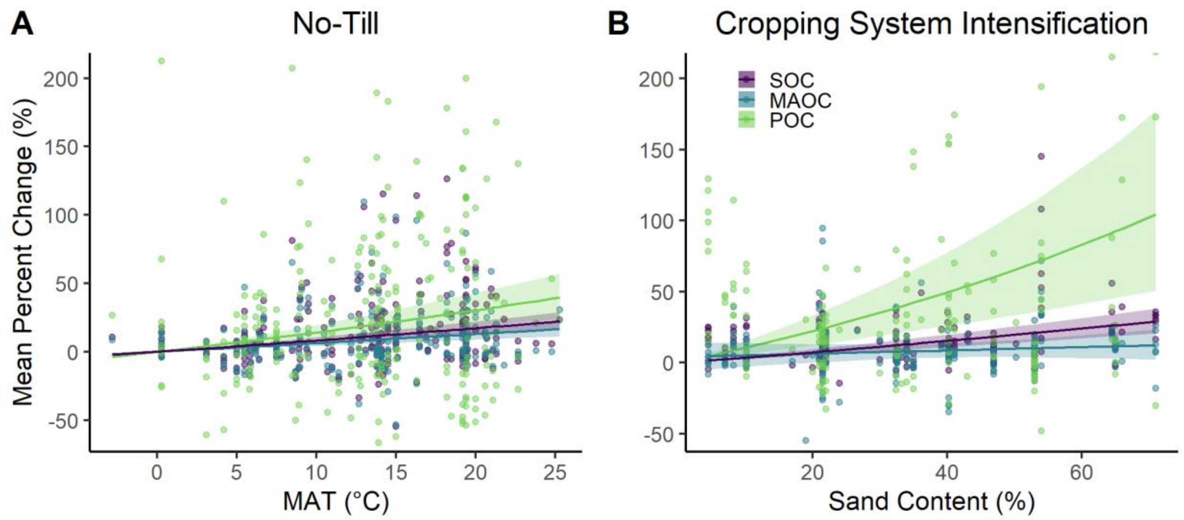

**Figure 2.6. A**, Meta-regression of mean annual temperature (MAT °C) against mean response of no-till on total soil organic carbon (SOC), mineral-associated organic carbon (MAOC), and particulate organic carbon (POC) concentrations (g C kg soil$^{-1}$) in topsoil (0-20cm). **B**, Meta-regression of sand content (%) against mean response of cropping system intensification on SOC, MAOC, and POC in topsoil.

# 3. DISCUSSION

## *3.1 Overall effects of regenerative agriculture on SOC pools and relationship to stocks*

Our global synthesis provides clear evidence for the ability of regenerative practices to increase both MAOC and POC in topsoil (0-20 cm) compared to conventional management. As MAOC was the larger fraction (~80% of C) in all soils, any changes to this fraction correlated with changes in total SOC. The absolute response of POC to regenerative practices was smaller and more variable than that of MAOC, suggesting that moderating factors are more important in governing the persistence and stabilization of POC compared to MAOC under regenerative management. MAOC is often positioned as a target for soil C sequestration due to its longer turnover times (Poeplau et al., 2018). Our results suggest that it is in fact an appropriate target also because of its larger absolute increases under regenerative management. While POC absolute increases are of minor importance to SOC gains (Fig. 2.1), this analysis pointed to a few practices having high effect on POC accrual (>50% increases), indicating their potential on the long-term to regenerate in cropland soils to a more balanced distribution of C in POC and MAOC, typical of natural soils (Lugato et al., 2021).

Practitioners of regenerative practices are unlikely to see the positive benefits to SOC (POC or MAOC) until after six years of implementation, and in many instances may see significant decreases in SOC before accumulation occurs (Fig. 2.3 & 2.4). Monitoring for fast (1-5 years) SOC stock changes under regenerative agriculture should thus not be used to draw conclusions about long-term SOC response.

We did not observe decreases in subsoil (20-100 cm) C due to regenerative management, as has been occasionally observed with no-till (Luo et al., 2010). In fact, in many instances, POC was significantly increased in the subsoil from cropping intensification, especially with the inclusion of perennials (Fig. 2.2, Supplementary Fig. 2.1). While there is evidence of publication bias favoring reporting of positive, significant results, the skew is very slight and likely only minorly impacts our results (Supplementary Fig. 2.2).

### *3.2 SOC changes under no-till*

We hypothesized that no-till would primarily increase POC due to its effect on maintaining soil aggregates, an acknowledged mechanism of POC protection (Six et al., 1999; Mondal et al., 2020). However, we found that, in topsoil, no-till increases MAOC as well as POC. No-till may alter quantity and distribution of C inputs through roots. After initial yield decreases due to soil compaction (de Moura et al., 2021), no-till may increase root C inputs, a strong pathway for MAOC formation (Fulton-Smith and Cotrufo, 2019; Sokol et al., 2019). Alternatively, differences in POC and MAOC could be the direct result of reduced C losses by erosion in NT systems (Lee et al., 2021).

Surprisingly, the frequency of tillage did not moderate the effect of tillage on POC, possibly because any amount of tillage was enough to disrupt aggregate formation and prevent POC accumulation. MAOC stabilization, however, was greatly affected by the frequency of tillage. This is possibly a result of chronic disturbance which may prevent the recovery of fungal networks and other soil organisms, such as earthworms (Briones and Schmidt, 2017), which have shown to be critical for stable SOC formation (Angst et al., 2019; Liang et al., 2019).

The negative effects of tillage on POC are mitigated by intensifying the cropping system, suggesting that in regenerative systems occasional tillage events will not greatly affect POC stocks. In contrast, cropping system intensity had a synergistic effect with NT on MAOC with MAOC increases from NT being greatest in systems with multiple crops per year. This is possibly because intact fungal networks are able to efficiently process the continuous plant inputs to form MAOC (Wei et al., 2022).

### *3.3 SOC changes under cropping system intensification*

Consistent with our expectations, cropping system intensification increased both MAOC and POC. With cropping system intensification, C increases are likely the result of increased C inputs( King and Blesh, 2018), and established pathways link POC formation with plant structural residues and MAOC formation with plant exudation through living roots( Fulton-Smith and Cotrufo, 2019).

We found the most important method of intensification for increasing POC was the elimination of summer fallow with continuous cropping. Additional intensification with multiple crops per year did not increase POC further, although intensifying continuous cropping with cover crops and perennials did further increase POC. Differences in quantity or quality of belowground inputs with cash crop vs. cover crops or perennial crops may explain this apparent discrepancy. Perennial intensification was especially important for increasing POC as this was the only practice that also increased POC in both subsoil and topsoil. This is likely due to the greater and deeper root system in perennials as compared to annuals (Husáková et al., 2018).

The benefit of intensification on POC and MAOC was lessened as diversity increased. Common choices in research site design may help to explain this finding, as systems that were intensified using perennials often did not increase in diversity (e.g., corn-soy intensified to corn-alfalfa-alfalfa-alfalfa). Similarly, more diverse systems containing summer fallow were frequently compared to continuous annual monoculture (e.g., corn-soy-fallow compared to continuous corn). MAOC formation appears to depend less on diversifying the rotation than on reducing fallow to maintain continuous inputs.

Finally, cropping intensification had a greater effect in CT than in NT systems, especially for MAOC. The explanation could be that SOC concentrations in CT systems are lower than in NT systems, therefore, the same SOC absolute change due to intensification will be proportionally larger under CT; or that in heavily disturbed CT systems, intensification may reduce bare ground minimizing loss of SOC and nutrients through erosion (Komissarov and Klik, 2020).

### *3.4 Potential synergies within integrated crop-livestock systems*

As only a limited number of studies explored combinations of regenerative practices with ICL, we consider our results compelling preliminary evidence, with further research needed to provide unequivocal support of synergies. Nevertheless, our results show an enormous potential for ICL to increase SOC, POC, and MAOC, primarily when used in combination with NT and cropping intensification. When ICL was used in combination with NT, we found significant POC increases that exceeded those from NT alone indicating an additive interaction likely through enhanced aggregation and improved soil structure (Fultz et al., 2013; Bansal et al., 2022).

We also found evidence of synergy between ICL and cropping system intensification. Though ICL and cover crop together and cover crop alone both raised total SOC to the same extent, the distribution of C increase shifted from POC to MAOC when livestock were included. The synergy between ICL and perennial intensification on MAOC was even more pronounced, yet the variation in effect sizes makes it difficult to draw firm conclusions about the effect on POC. Positive synergies between ICL and cropping system intensification on SOC may occur due to impacts of grazing on root photosynthate allocation (Chen et al., 2015) and microbial decomposition through changes to C-to-N ratios of plant litter and the addition of animal waste (Piñeiro et al., 2009; Tian et al., 2010).

### *3.5 Regenerative practices effective across a range of soil and climate conditions*

Environmental moderators explained a minor portion of the variance in effect sizes of regenerative management on SOC, MAOC, and POC. The only environmental variable to significantly moderate the effect of NT was MAT. A greater relative effect of NT compared to CT on POC and MAOC in warm climates may be due to a synergistic interaction between disturbance due to tillage and overall high microbial activity in warmer climates. In other words, when microbes are inhibited by cooler temperatures, soil disturbance would not stimulate additional decomposition (Cotrufo and Lavallee, 2022).

Across environmental moderators, only sand content significantly moderated the effect of cropping intensification on POC, but not MAOC. Since MAOC formation depends on the availability of binding sites in the silt/clay mineral matrix, we expected a negative relationship between percent sand and MAOC gains. However, a recent analysis has shown that agricultural systems are only at ~31% MAOC saturation (Georgiou et al., 2022), and indeed we found no evidence for a constraint on MAOC formation in soils with low silt/clay content.

### *3.6 Limitations and next steps*

Our meta-analysis was constrained by data available in the literature. Although SOC stock change with management can inform about C budgeting, fewer than half the studies reported information needed to calculate SOC stocks, so we focused on C concentrations. The lack of baseline SOC data prevented conclusions about whether true C sequestration, emissions avoided, or a combination of the two, were observed with soil C change. Average soil sampling depth in our database was 20 cm, and future studies will enable a more comprehensive understanding of soil C if reported across the soil profile. Finally, though all major continents are represented in our analysis, our database revealed data gaps in South America, Africa, Eurasia, and Oceania (Supplementary Fig. 2.3).

This analysis shows that crop intensification and no-till increase POC and MAOC, thus both improving soil health and promoting long-term carbon stabilization. However, the optimization of stacked management strategies to enhance POC and MAOC still has specific knowledge gaps, including the mechanisms driving observed synergies in the integration of livestock into cropland in combination with other regenerative practices. Developing a comprehensive understanding of these and other synergies is essential for maximizing regenerative agriculture's ability to mitigate climate change and provide additional ecosystem services to benefit producers, the environment, and society at large.

## 4. METHODS

### *4.1 Study selection*

We searched Web of Science and Agricultural Online Access Database (AGRICOLA) to conduct a systematic meta-analysis on the response of SOC pools to tillage management, cropping intensification, and integrated-crop livestock management (ICL) (see Supplementary Table 2.3 for list of Boolean search terms). The search included all papers published until January 2021. After removal of duplicates, our search yielded 4,706 publications. We screened publications following PRISMA guidelines (Moher et al., 2009) using the following criteria: (1) peer-reviewed, primary literature; (2) measured SOC fractions that are or can be combined to MAOC and POC; (3) measured response of POC and/or MAOC to soil management intervention with a control that was not treated with that intervention; (4) experimental treatments must include one of these soil management practices: tillage management, cropping intensification management, and integrated livestock management. Studies were excluded if the treatment of interest was not isolated within the experimental design (e.g., when corn-soybean crop rotations w/ fertilizer are compared to corn-soybean-wheat-winter rye rotations w/o fertilizer the causal effects of rotation intensification cannot be disentangled from fertilization treatment).

To be included in this study, at least three replicates of SOC, MAOC, and/or POC needed to be measured to ensure robustness of the dataset. Soil had to be sieved to 2mm or less and dispersed using either sodium hexametaphosphate, sonication, or shaking with glass beads such that aggregates would be adequately broken down. For this study, MAOC was defined as the fraction of SOC smaller than 50-63 µm, when separated by size, or heavier than 1.6-1.85g $cm^{-3}$, when separated by density (Leuthold et al., 2022). POC was defined as the coarse (>50-63 µm) and/or light (<1.6-1.85g $cm^{-3}$) fraction, the counterpart to MAOC. Heavy, coarse SOC fractions resulting from the combination of size and density fractionation (i.e >1.6 g $cm^{-3}$ and > 50–63 µm) were considered MAOC due to having low C:N similar to the fine, heavy fraction(Samson et al., 2020). Only studies that directly quantified C using direct combustion or wet oxidation methods were included.

### *4.2 Data collection and management*

In total, we identified 139 studies, 187 experiments, and 1250 observations across the world (Supplementary Fig. 2.3) that met our inclusion criteria. A complete list of publications included in the analysis can be found in SI References.

We extracted information on C concentrations, percent organic C, and C stocks. If only total SOC and one of the pools was measured, the other was calculated by subtraction. If free and occluded POC content were reported in a study, we summed them as POC content. If different size MAOC content were reported in a study, we summed them as MAOC content. If C concentrations were reported on per aggregate mass basis, we converted to per soil mass (g C $kg^{-1}$ of soil). We included data under control and treatment at last sampling time point if the soils were collected at different times. We extracted data from tables using Tabula software (https://tabula.technology/). If data were only presented in figures, *metaDigitize* package in R (Pick et al., 2019) was used for the extraction of data.

For the moderator analyses, we extracted information on agronomic factors including experimental duration, tillage frequency (tillage events per year), and cropping system. In addition, we extracted information on soil sampling depth, soil properties (e.g., bulk density, soil texture, percent sand/silt/clay, soil pH, and initial concentration of SOC), and climate characteristics (i.e., mean annual precipitation [MAP], mean annual temperature [MAT], and lat/long). If percent sand/silt/clay were not reported, we converted soil texture classification into percent sand/silt/clay using the USDA soil texture calculator. If lat/long were not reported, we estimated coordinates by searching site name on Google Earth. If climate characteristics were not reported, we extracted MAT and MAP from WorldClim v2.1 (Fick and Hijmans, 2017). Potential evapotranspiration (PET) data were extracted from the Global Aridity Index and Potential Evapotranspiration Database (Zomer et al., 2022).

Cropping system was characterized based on total number of species in rotation (diversity), crop species identities (corn, soy, etc.), frequency of crop occurrence, crop functional group (e.g., grass, legume, other), crop life history (e.g., annual or perennial), and crop residue management (e.g., grain harvest, hay harvest, no harvest). Frequency of crop occurrence was calculated as the number of times a crop appeared in rotation divided by the rotation years. Cropping system intensity was calculated as sum of all crop frequencies. For example, the cropping system intensity of a four-year rotation of corn-soy-corn-wheat would be calculated as $^{2}/_{4}$ (corn) + $^{1}/_{4}$ (soy) + $^{1}/_{4}$ (wheat) = 1. Cover crops were counted the same as other crops. Perennial crops were given an occurrence of 2 for each year in rotation since the crop occurred during both spring/summer and summer/fall growing seasons. For example, the cropping system intensity of a four-year rotation of corn-alfalfa-alfalfa-alfalfa would be calculated as $^{1}/_{4}$ (corn) +

1 $^{1}/_{2}$ (alfalfa) = 1 $^{3}/_{4}$. Effect sizes for cropping intensification were calculated comparing systems with an intensity equal to 1 (one crop per year) to those with an intensity < 1 (crop-fallow) and systems with an intensity > 1 (multiple crops per year) to rotations with an intensity ≤ 1. Diversity moderator categories were determined based on the difference in diversity between the more intense and less intense cropping system. Three diversity moderator categories were used: i) intensification with no change in diversity (monoculture), ii) intensification with an increase in diversity of two species (biculture), or iii) intensification with an increase of more than two species (polyculture).

### *4.3 Data analysis*

We chose C concentration (g C kg soil$^{-1}$) as the response variable for SOC, MAOC, and POC over C stock (Mg C ha$^{-1}$) because 44% of the studies did not report stock or bulk density values. If stock and bulk density was reported, we calculated concentration using the following equation:

$$C_c = 10 * \ C_s/(BD * depth) \tag{1}$$

where $C_c$ is the C concentration in g C kg soil$^{-1}$, $C_s$ is the C stock in Mg C ha$^{-1}$, *BD* is the bulk density in g cm$^{-3}$, and *depth* is the soil sampling depth increment in cm.

Variance data were frequently not reported. Instead of excluding these publications (28 publications, 219 observations - 18% of total) from analysis, which could introduce bias, we imputed missing standard deviations using the following equation (Bracken, 1992):

$$\widetilde{SD}_j = \bar{X}_j\left(\frac{\sum_i^K SD_i}{\sum_i^K \bar{X}_i}\right) \tag{2}$$

where $\bar{X}_j$ is the observed mean of the study with missing SD, and K is the number of *j*th studies with SD.

We estimated the effect size of SOC, MAOC, and POC for tillage treatment, cropping intensification, and ITL using the *metafor* package (Viechtbauer, 2010) in R ver 4.2.1. The effect size was calculated as:

$$\ln RR = \ln\left(\frac{\bar{X}_{trt}}{\bar{X}_{ctrl}}\right) \qquad (3)$$

where ln*RR* is the natural log of the response ratio, $\bar{X}_{trt}$ is the mean value of regenerative practice (i.e., no-till, cropping intensification, livestock integration), and $\bar{X}_{ctrl}$ is the mean value of the conventional control. To improve interpretation, we transformed these values to mean percent change (MPC) using the following equation:

$$Mean\ Percent\ Change\ = \left(e^{\ln RR} - 1\right) * 100\% \qquad (4)$$

We applied a three-level multivariate approach using the rma.mv function in *metafor* package to account for dependency of effect sizes. The model structure accounts for three levels of variance: (1) sampling variance of individual effect sizes, (2) variance between effect sizes from the same study, and (3) variance between studie s(Assink and Wibbelink, 2016). To account for the level of precision between studies, individual effect size was weighted by the inverse of the sampling variance(Hedges and Olkin, 2014).

We used log likelihood-ratio tests to evaluate homogeneity of within-study and between-study variance and found significant variation across effect sizes (Assink and Wibbelink, 2016). We tested each individual moderator with each regenerative intervention-SOC pool pairing and performed pairwise comparisons of each level of moderator using an omnibus test of model coefficients. To determine robustness of these relationships, we added all significant moderators into one model. We considered the relationships robust if moderators were still significant following the multiple moderator test (Assink and Wibbelink, 2016).

We tested for publication bias using Egger's regression test by including standard error of the effect sizes as a moderator (Habeck and Schultz, 2015). The relationship between the precision and size of studies is considered asymmetrical, and therefore biased, when the intercept of this regression test significantly deviates from zero ($p < 0.1$) (Egger et al., 1997). To further evaluate publication bias, we visually assessed histograms of lnRR for left or right skew (Supplementary Fig. 2.2). Histograms of the overall log response ratio (lnRR) provided evidence for a lack of publication bias (Supplementary Fig. 2.2). However, Egger's test indicated slight overestimation of effect sizes for SOC (int = 0.111, $p < 0.001$), MAOC (int = 0.059, $p < 0.001$), and POC (int = 0.278, $p < 0.001$) in response to regenerative management.

We conducted a sensitivity analysis to assess the robustness of the overall results. To quantify the impact of each eliminated study, we employed a jackknife approach to remove specific studies from each regenerative intervention-SOC pool pair and recalculate the mean effect sizes(Basche and DeLonge, 2019) (Supplementary Fig. 2.4). The sensitivity analysis indicated these results were robust and that no particular publication drove the overall response (Supplementary Fig. 2.4).

## REFERENCES

Abdalla, M., Hastings, A., Cheng, K., Yue, Q., Chadwick, D., Espenberg, M., Truu, J., Rees, R.M., Smith, P., 2019. A critical review of the impacts of cover crops on nitrogen leaching, net greenhouse gas balance and crop productivity. Global Change Biology 25, 2530–2543. doi:10.1111/gcb.14644

Acosta-Martínez, V., Zobeck, T.M., Allen, V., 2004. Soil Microbial, Chemical and Physical Properties in Continuous Cotton and Integrated Crop–Livestock Systems. Soil Science Society of America Journal 68, 1875–1884. doi:10.2136/sssaj2004.1875

Angst, G., Mueller, C.W., Prater, I., Angst, Š., Frouz, J., Jílková, V., Peterse, F., Nierop, K.G.J., 2019. Earthworms act as biochemical reactors to convert labile plant compounds into stabilized soil microbial necromass. Communications Biology 2, 1–7. doi:10.1038/s42003-019-0684-z

Assink, M., Wibbelink, C.J.M., 2016. Fitting three-level meta-analytic models in R: A step-by-step tutorial. The Quantitative Methods for Psychology 12, 154–174. doi:10.20982/tqmp.12.3.p154

Bansal, S., Chakraborty, P., Kumar, S., 2022. Crop–livestock integration enhanced soil aggregate-associated carbon and nitrogen, and phospholipid fatty acid. Scientific Reports 12, 2781. doi:10.1038/s41598-022-06560-6

Basche, A.D., DeLonge, M.S., 2019. Comparing infiltration rates in soils managed with conventional and alternative farming methods: A meta-analysis. PLOS ONE 14, e0215702. doi:10.1371/journal.pone.0215702

Bracken, M.B., 1992. Statistical methods for analysis of effects of treatment in overviews of randomized trials, in: Effective Care of the Newborn Infant (Eds J.C. Sinclair and M.B. Bracken). Oxford University Press, Oxford, pp. 13–20.

Brewer, K.M., Gaudin, A.C.M., 2020. Potential of crop-livestock integration to enhance carbon sequestration and agroecosystem functioning in semi-arid croplands. Soil Biology and Biochemistry 149, 107936. doi:10.1016/j.soilbio.2020.107936

Briones, M.J.I., Schmidt, O., 2017. Conventional tillage decreases the abundance and biomass of earthworms and alters their community structure in a global meta-analysis. Global Change Biology 23, 4396–4419. doi:10.1111/gcb.13744

Chen, W., Huang, D., Liu, N., Zhang, Y., Badgery, W.B., Wang, X., Shen, Y., 2015. Improved grazing management may increase soil carbon sequestration in temperate steppe. Scientific Reports 5, 10892. doi:10.1038/srep10892

Conant, R.T., Cerri, C.E.P., Osborne, B.B., Paustian, K., 2017. Grassland management impacts on soil carbon stocks: a new synthesis. Ecological Applications 27, 662–668. doi:10.1002/eap.1473

Cotrufo, M.F., Lavallee, J.M., 2022. Chapter One - Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 1–66. doi:10.1016/bs.agron.2021.11.002

Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. Nature Geoscience 12, 989–994. doi:10.1038/s41561-019-0484-6

Crews, T.E., Rumsey, B.E., 2017. What agriculture can learn from native ecosystems in building soil organic matter: A review. Sustainability 9, 578. doi:10.3390/su9040578 de Moura, M.S., Silva, B.M., Mota, P.K., Borghi, E., Resende, A.V. de, Acuña-Guzman, S.F., Araújo, G.S.S., da Silva, L. de C.M., de Oliveira, G.C., Curi, N., 2021. Soil management and diverse crop rotation can mitigate early-stage no-till compaction and improve least limiting water range in a Ferralsol. Agricultural Water Management 243, 106523. doi:10.1016/j.agwat.2020.106523

Egger, M., Smith, G.D., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test. BMJ 315, 629–634. doi:10.1136/bmj.315.7109.629

Fick, S.E., Hijmans, R.J., 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37, 4302–4315. doi:10.1002/joc.5086

Franzluebbers, A.J., 2010. Achieving soil organic carbon sequestration with conservation agricultural systems in the southeastern United States. Soil Science Society of America Journal 74, 347–357. doi:10.2136/sssaj2009.0079

Fulton-Smith, S., Cotrufo, M.F., 2019. Pathways of soil organic matter formation from above and belowground inputs in a Sorghum bicolor bioenergy crop. GCB Bioenergy 11, 971–987. doi:10.1111/gcbb.12598

Fultz, L.M., Moore-Kucera, J., Zobeck, T.M., Acosta-Martínez, V., Allen, V.G., 2013. Aggregate Carbon Pools after 13 Years of Integrated Crop-Livestock Management in Semiarid Soils. Soil Science Society of America Journal 77, 1659–1666. doi:10.2136/sssaj2012.0423

Georgiou, K., Jackson, R.B., Vindušková, O., Abramoff, R.Z., Ahlström, A., Feng, W., Harden, J.W., Pellegrini, A.F.A., Polley, H.W., Soong, J.L., Riley, W.J., Torn, M.S., 2022. Global stocks and capacity of mineral-associated soil organic carbon. Nature Communications 13, 3797. doi:10.1038/s41467-022-31540-9

Guo, L.B., Gifford, R.M., 2002. Soil carbon stocks and land use change: a meta analysis. Global Change Biology 8, 345–360. doi:10.1046/j.1354-1013.2002.00486.x

Habeck, C.W., Schultz, A.K., 2015. Community-level impacts of white-tailed deer on understorey plants in North American forests: a meta-analysis. AoB PLANTS 7, plv119. doi:10.1093/aobpla/plv119

Hassink, J., 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant and Soil 191, 77–87. doi:10.1023/A:1004213929699

Hedges, L.V., Olkin, I., 2014. Statistical methods for meta-analysis. Academic Press, Cambridge, Massachusetts, USA.

Husáková, I., Weiner, J., Münzbergová, Z., 2018. Species traits and shoot–root biomass allocation in 20 dry-grassland species. Journal of Plant Ecology 11, 273–285. doi:10.1093/jpe/rtw143

Jayaraman, S., Sinha, N.K., Kumar, S., Patra, A.K., 2021. Editorial: Sustaining soil carbon to enhance soil health, food, nutritional security, and ecosystem services. Frontiers in Sustainable Food Systems 5.

Jordon, M.W., Smith, P., Long, P.R., Bürkner, P.-C., Petrokofsky, G., Willis, K.J., 2022. Can Regenerative Agriculture increase national soil carbon stocks? Simulated country-scale adoption of reduced tillage, cover cropping, and ley-arable integration using RothC. Science of The Total Environment 825, 153955. doi:10.1016/j.scitotenv.2022.153955

Just, C., Poeplau, C., Don, A., van Wesemael, B., Kögel-Knabner, I., Wiesmeier, M., 2021. A simple approach to isolate slow and fast cycling organic carbon fractions in central European soils—Importance of dispersion method. Frontiers in Soil Science 1.

King, A.E., Blesh, J., 2018. Crop rotations for increased soil carbon: perenniality as a guiding principle. Ecological Applications 28, 249–261. doi:10.1002/eap.1648

Komissarov, M.A., Klik, A., 2020. The impact of no-till, conservation, and conventional tillage systems on erosion and soil properties in lower Austria. Eurasian Soil Science 53, 503–511. doi:10.1134/S1064229320040079

Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304, 1623–1627. doi:10.1126/science.1097396

Lavallee, J.M., Soong, J.L., Cotrufo, M.F., 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. Global Change Biology 26, 261–273. doi:10.1111/gcb.14859

Ledo, A., Smith, P., Zerihun, A., Whitaker, J., Vicente-Vicente, J.L., Qin, Z., McNamara, N.P., Zinn, Y.L., Llorente, M., Liebig, M., Kuhnert, M., Dondini, M., Don, A., Diaz-Pines, E., Datta, A., Bakka, H., Aguilera, E., Hillier, J., 2020. Changes in soil organic carbon under perennial crops. Global Change Biology 26, 4158–4168. doi:10.1111/gcb.15120

Lee, S., Chu, M.L., Guzman, J.A., Botero-Acosta, A., 2021. A comprehensive modeling framework to evaluate soil erosion by water and tillage. Journal of Environmental Management 279, 111631. doi:10.1016/j.jenvman.2020.111631

Lemaire, G., Franzluebbers, A., Carvalho, P.C. de F., Dedieu, B., 2014. Integrated crop–livestock systems: Strategies to achieve synergy between agricultural production and environmental quality. Agriculture, Ecosystems & Environment, Integrated Crop-Livestock System Impacts on Environmental Processes 190, 4–8. doi:10.1016/j.agee.2013.08.009

Leuthold, S.J., Haddix, M.L., Lavallee, J., Cotrufo, M.F., 2022. Physical fractionation techniques, in: Reference Module in Earth Systems and Environmental Sciences. Elsevier. doi:10.1016/B978-0-12-822974-3.00067-7

Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Global Change Biology 25, 3578–3590. doi:10.1111/gcb.14781

Lugato, E., Lavallee, J.M., Haddix, M.L., Panagos, P., Cotrufo, M.F., 2021. Different climate sensitivity of particulate and mineral-associated soil organic matter. Nature Geoscience 14, 295–300. doi:10.1038/s41561-021-00744-x

Luo, Z., Wang, E., Sun, O.J., 2010. Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. Agriculture, Ecosystems & Environment 139, 224–231. doi:10.1016/j.agee.2010.08.006

McClelland, S.C., Paustian, K., Schipanski, M.E., 2021. Management of cover crops in temperate climates influences soil organic carbon stocks: a meta-analysis. Ecological Applications 31, e02278. doi:10.1002/eap.2278

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecological Applications 24, 560–570. doi:10.1890/13-0616.1

Minasny, B., Malone, B.P., McBratney, A.B., Angers, D.A., Arrouays, D., Chambers, A., Chaplot, V., Chen, Z.-S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C.B., Hong, S.Y., Mandal, B., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovoy, V., Stockmann, U., Sulaeman, Y., Tsui, C.-C., Vågen, T.-G., van Wesemael, B., Winowiecki, L., 2017. Soil carbon 4 per mille. Geoderma 292, 59–86. doi:10.1016/j.geoderma.2017.01.002

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 339, b2535. doi:10.1136/bmj.b2535

Mondal, S., Chakraborty, D., Bandyopadhyay, K., Aggarwal, P., Rana, D.S., 2020. A global analysis of the impact of zero-tillage on soil physical condition, organic carbon content, and plant root response. Land Degradation & Development 31, 557–567. doi:10.1002/ldr.3470

Ogle, S.M., Breidt, F.J., Paustian, K., 2005. Agricultural management impacts on soil organic carbon storage under moist and dry climatic conditions of temperate and tropical regions. Biogeochemistry 72, 87–121. doi:10.1007/s10533-004-0360-2

Ogle, S.M., Swan, A., Paustian, K., 2012. No-till management impacts on crop productivity, carbon input and soil carbon sequestration. Agriculture, Ecosystems & Environment 149, 37–49. doi:10.1016/j.agee.2011.12.010

Oldfield, E.E., Bradford, M.A., Wood, S.A., 2019. Global meta-analysis of the relationship between soil organic matter and crop yields. SOIL 5, 15–32. doi:10.5194/soil-5-15-2019

Pan, G., Smith, P., Pan, W., 2009. The role of soil organic matter in maintaining the productivity and yield stability of cereals in China. Agriculture, Ecosystems & Environment 129, 344–348. doi:10.1016/j.agee.2008.10.008

Paul, E.A., 2016. The nature and dynamics of soil organic matter: Plant inputs, microbial transformations, and organic matter stabilization. Soil Biology and Biochemistry 98, 109–126. doi:10.1016/j.soilbio.2016.04.001

Paul, E.A., Elliott, E.T., Paustian, K., Cole, C.V. (Eds.), 2019. Soil Organic Matter in Temperate Agroecosystems: Long-Term Experiments in North America. CRC Press, Boca Raton. doi:10.1201/9780367811693

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G.P., Smith, P., 2016. Climate-smart soils. Nature 532, 49–57. doi:10.1038/nature17174

Pick, J.L., Nakagawa, S., Noble, D.W.A., 2019. Reproducible, flexible and high-throughput data extraction from primary literature: The metaDigitise r package. Methods in Ecology and Evolution 10, 426–431. doi:10.1111/2041-210X.13118

Piñeiro, G., Paruelo, J.M., Jobbágy, E.G., Jackson, R.B., Oesterheld, M., 2009. Grazing effects on belowground C and N stocks along a network of cattle exclosures in temperate and subtropical grasslands of South America. Global Biogeochemical Cycles 23. doi:10.1029/2007GB003168

Poeplau, C., Don, A., 2015. Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. Agriculture, Ecosystems & Environment 200, 33–41. doi:10.1016/j.agee.2014.10.024

Poeplau, C., Don, A., Six, J., Kaiser, M., Benbi, D., Chenu, C., Cotrufo, M.F., Derrien, D., Gioacchini, P., Grand, S., Gregorich, E., Griepentrog, M., Gunina, A., Haddix, M., Kuzyakov, Y., Kühnel, A., Macdonald, L.M., Soong, J., Trigalet, S., Vermeire, M.-L., Rovira, P., van Wesemael, B., Wiesmeier, M., Yeasmin, S., Yevdokimov, I., Nieder, R., 2018. Isolating organic carbon fractions with varying turnover rates in temperate agricultural soils – A comprehensive method comparison. Soil Biology and Biochemistry 125, 10–26. doi:10.1016/j.soilbio.2018.06.025

Rosenzweig, S.T., Fonte, S.J., Schipanski, M.E., 2018. Intensifying rotations increases soil carbon, fungi, and aggregation in semi-arid agroecosystems. Agriculture, Ecosystems & Environment 258, 14–22. doi:10.1016/j.agee.2018.01.016

Samson, M.-É., Chantigny, M.H., Vanasse, A., Menasseri-Aubry, S., Angers, D.A., 2020. Coarse mineral-associated organic matter is a pivotal fraction for SOM formation and is sensitive to the quality of organic inputs. Soil Biology and Biochemistry 149, 107935. doi:10.1016/j.soilbio.2020.107935

Sanderman, J., Hengl, T., Fiske, G.J., 2017. Soil carbon debt of 12,000 years of human land use. Proceedings of the National Academy of Sciences 114, 9575–9580. doi:10.1073/pnas.1706103114

Schreefel, L., Schulte, R.P.O., de Boer, I.J.M., Schrijver, A.P., van Zanten, H.H.E., 2020. Regenerative agriculture – the soil is the base. Global Food Security 26, 100404. doi:10.1016/j.gfs.2020.100404

Six, J., Elliott, E.T., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Science Society of America Journal 63, 1350–1358. doi:10.2136/sssaj1999.6351350x

Sokol, N.W., Kuebbing, S.E., Karlsen-Ayala, E., Bradford, M.A., 2019. Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon. The New Phytologist 221, 233–246. doi:10.1111/nph.15361

Tian, L., Dell, E., Shi, W., 2010. Chemical composition of dissolved organic matter in agroecosystems: Correlations with soil enzyme activity and carbon and nitrogen mineralization. Applied Soil Ecology 46, 426–435. doi:10.1016/j.apsoil.2010.09.007

Tracy, B.F., Zhang, Y., 2008. Soil compaction, corn yield response, and soil nutrient pool dynamics within an integrated crop-livestock system in Illinois. Crop Science 48, 1211–1218. doi:10.2135/cropsci2007.07.0390

Viechtbauer, W., 2010. Conducting Meta-Analyses in R with the metafor Package. Journal of Statistical Software 36, 1–48. doi:10.18637/jss.v036.i03

Wei, Y., Xiong, X., Ryo, M., Badgery, W.B., Bi, Y., Yang, G., Zhang, Y., Liu, N., 2022. Repeated litter inputs promoted stable soil organic carbon formation by increasing fungal dominance and carbon use efficiency. Biology and Fertility of Soils 58, 619–631. doi:10.1007/s00374-022-01647-8

Zomer, R.J., Bossio, D.A., Sommer, R., Verchot, L.V., 2017. Global sequestration potential of increased organic carbon in cropland soils. Scientific Reports 7, 15554. doi:10.1038/s41598-017-15794-8

Zomer, R.J., Xu, J., Trabucco, A., 2022. Version 3 of the Global Aridity Index and Potential Evapotranspiration Database. Scientific Data 9, 409. doi:10.1038/s41597-022-01493-1

# CHAPTER 3: EVALUATING THE IMPACT OF REGENERATIVE AGRICULTURAL SYSTEMS ON SOIL CARBON, SOIL HEALTH, AND YIELD: A POLICY BRIEF[2]

## 1. EXECUTIVE SUMMARY

The primary objective of this research was to evaluate the impacts of regenerative agricultural systems on soil carbon, soil health, and agronomic outcomes and how these outcomes vary across space and time. Regenerative agriculture is defined as a system the employs more than one USDA NRCS soil health principle This work has resulted in four major findings:

1. Regenerative agricultural systems are frequently associated with higher levels of SOC.
2. Most studies show that implementation of multiple practices for a period of ten years or more is necessary to support positive carbon impacts.
3. The efficacy of practices in maintaining or enhancing carbon stocks varies by region.
4. Regenerative agricultural systems support higher yields and better soil health, including drought resilience, nutrient cycling, resistance to erosion, and biodiversity.

These findings give rise to the following recommendations:

1. Increase funding for practices that support soil health and positive carbon outcomes under the Conservation Stewardship Program and the Environmental Quality Incentives Program;
2. Support longer participation times in USDA NRCS conservation programs;
3. Tailor conservation programs regionally; and
4. Fund research and other activities to fill critical information gaps using regionally representative research farms.

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[2] In preparation to be published as a policy brief with Helen Silver, Megan Machmuller, and Jane Zelikova. Project funded by Breakthrough Strategies & Solutions, Regenerative Agriculture Foundation, and Panta Rhea Foundation

## 2. INTRODUCTION

A robust and resilient agricultural sector is essential for maintaining food security amidst accelerating climate risks. The USDA NRCS has established four key principles for soil health: minimizing disturbance (physical, chemical, and biological), maximizing the presence of living roots, maximizing soil cover, and maximizing biodiversity, including the integration of livestock. Depending on implementation, a single soil health practice may touch on one or more of these principles. For instance, cover crops terminated by crimp rolling may more effectively increase soil cover and minimize disturbance compared to cover crops terminated with chemicals. Determining appropriate practices to implement these principles must be conducted on a case-by-case basis for the crop or grazing land in question.

Research and farmer experience demonstrate that soil health practices both maintain and build soil organic carbon (SOC) stocks in the soil and enhance other metrics of soil health, including soil biodiversity, soil structure (an indicator of resistance to erosion and drought resilience), nutrient provisioning, and water infiltration (Tahat et al., 2020; Tully and McAskill, 2020; Yang et al., 2020). These principles and practices are generally considered a synergistic system, and optimizing all principles may require implementing multiple practices.

In recent years, the overall adoption of soil health practices has increased. According to the USDA, no-till is practiced on approximately 35% of total cropland acres, reduced tillage on 32%, and cover crops on 5.9% [3]. These practices have contributed to significant progress in maintaining the health of our crop and grazing lands. For instance, between 1982 and 2017, total erosion on croplands decreased from nearly 3 billion tons annually to less than 2 billion tons annually (USDA, "Workbook: RCA DV Erosion by State NRI 2017 1,"). These metrics demonstrate that while NRCS programs have been effective, significant room for improvement remains.

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[3] Excludes land enrolled in the Conserve Reserve Program.

Soil organic carbon is not only the single best indicator of general soil health, but measuring SOC stocks is also critical for estimating the climate change impacts of agricultural management practices. To understand climate impacts, changes to SOC stocks must be measured, which requires both SOC concentrations in a given unit of soil and calculating the bulk density (soil mass per volume) of soil. Compared to measuring SOC concentrations, relatively few studies calculate SOC stocks.

SOC accumulates, and management practices can influence SOC stocks at varying depths in the soil profile, sometimes up to one meter. SOC stocks are often measured at a depth of 15 cm if the primary research objective is to estimate impacts on soil health metrics, such as nutrient cycling and drought resilience. If the objective is to understand impacts on SOC stocks for climate mitigation purposes, SOC should be measured to a depth of at least 40 cm and may include depths of up to one meter.

Assuming accurate measurement of SOC stocks, increases in SOC between different management regimes may result from either carbon sequestration (drawdown of $CO_2$ from the atmosphere) or reduced SOC loss from the soil. Both scenarios have positive climate impacts, but only carbon sequestration directly draws down atmospheric $CO_2$ concentrations. Estimates of the potential climate impacts of carbon sequestration vary widely and are often attributable to differences in research design and methods in SOC measurement. However, significant gaps remain in understanding the impact of regenerative agricultural systems, which implement two or more soil health practices, compared to monocropped systems with conventional tillage.

Three important limitations exist concerning regenerative agricultural systems. First, the majority of studies focus solely on the impacts of an individual soil health practice. This is likely because examining a single practice significantly reduces the complexity of establishing causal and correlational relationships. Second, most research has focused on a few well-established practices, such as changes in tillage management and cover crops, though an abundance of evidence shows that other practices are critically important, including livestock integration, diversified rotations, and intercropping (Prairie et al., 2023). Finally, most research targets a few commodity crops, namely corn, soy, and wheat, likely because these crops are the largest economic drivers in the US food system and receive more funding.

In summary, climate risks are accelerating with potentially devastating impacts on US agricultural production, food security, and rural livelihoods. Further, research to date has been narrowly limited in terms of the number and types of soil health practices and crop types studied. While understudied, regenerative agricultural systems may significantly contribute to climate resilience. This study is a critical starting point for this evolving area of research and emphasizes the need for practical analyses to inform policy, justifying a less scientifically rigorous and broader impact approach.

The primary objective of this research is to evaluate the impacts of regenerative agricultural systems on soil carbon, soil health, and agronomic outcomes, and how these outcomes vary across different regions and time periods. This work addresses the need for practical analyses to inform policy, providing a broad understanding of the benefits and challenges associated with regenerative agricultural systems.

Due to constraints in the literature, our study makes no conclusions regarding whether regenerative agricultural systems show better carbon sequestration or drawdown than control systems. Instead, our results focus on overall soil carbon levels without distinguishing between sequestration or reduced SOC losses.

## 3. METHODS

### *3.1 Scoping criteria and record review*

To identify studies for inclusion, we developed several screening criteria. First, we defined regenerative agricultural systems as those systems that applied more than one regenerative agricultural practice simultaneously. Second, we limited our search to only those studies that evaluated impacts on SOC. Third, we restricted our research to published, peer-reviewed studies in the United States with at least three replicates and a valid control (Fig. 3.1). After rejecting several thousand studies, we reviewed a total of 146 studies[4] (Fig. 3.2; Table 3.1).

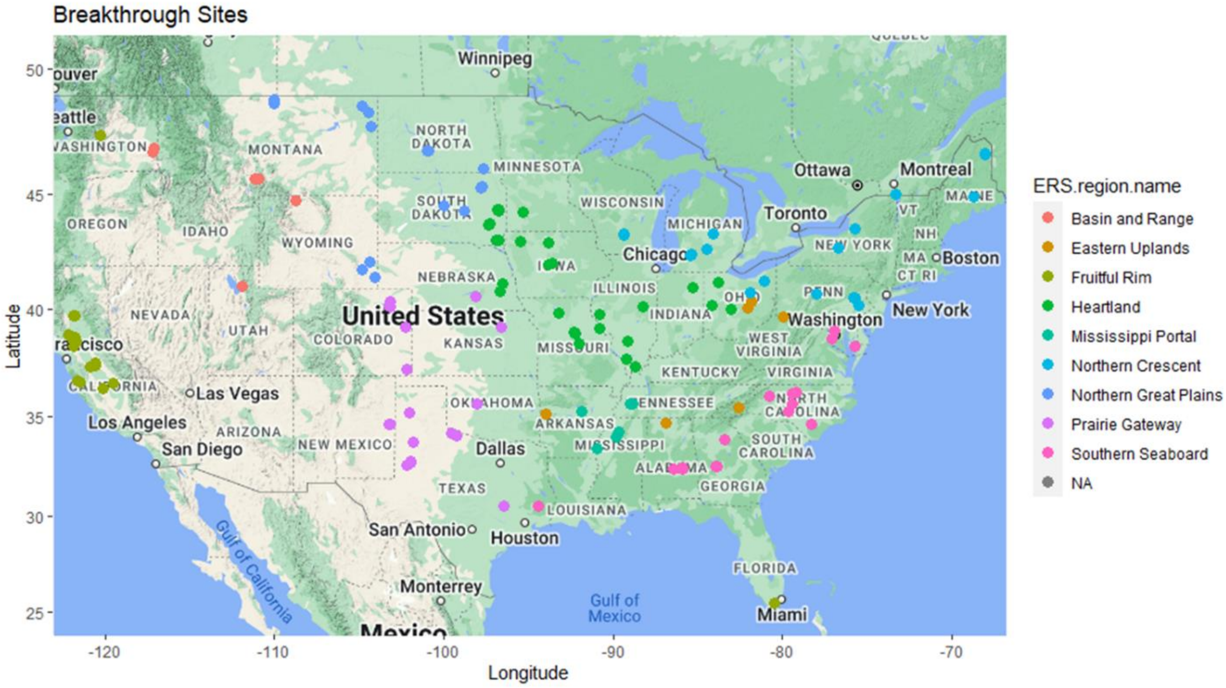

**Figure 3.1.** Map of studies used in quantitative literature review colored by USDA Farm Resource Region.

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[4] Of the original 6,992 records retrieved, we rejected 2,284 records as duplicative and screened the abstracts of 4708 records. Of those, 2747 were excluded because they did not meet the scoping criteria. An additional 48 were unavailable. The remaining 1,913 were read and a total of 1,767 were excluded. This left a total of 146 studies that met the screening criteria.

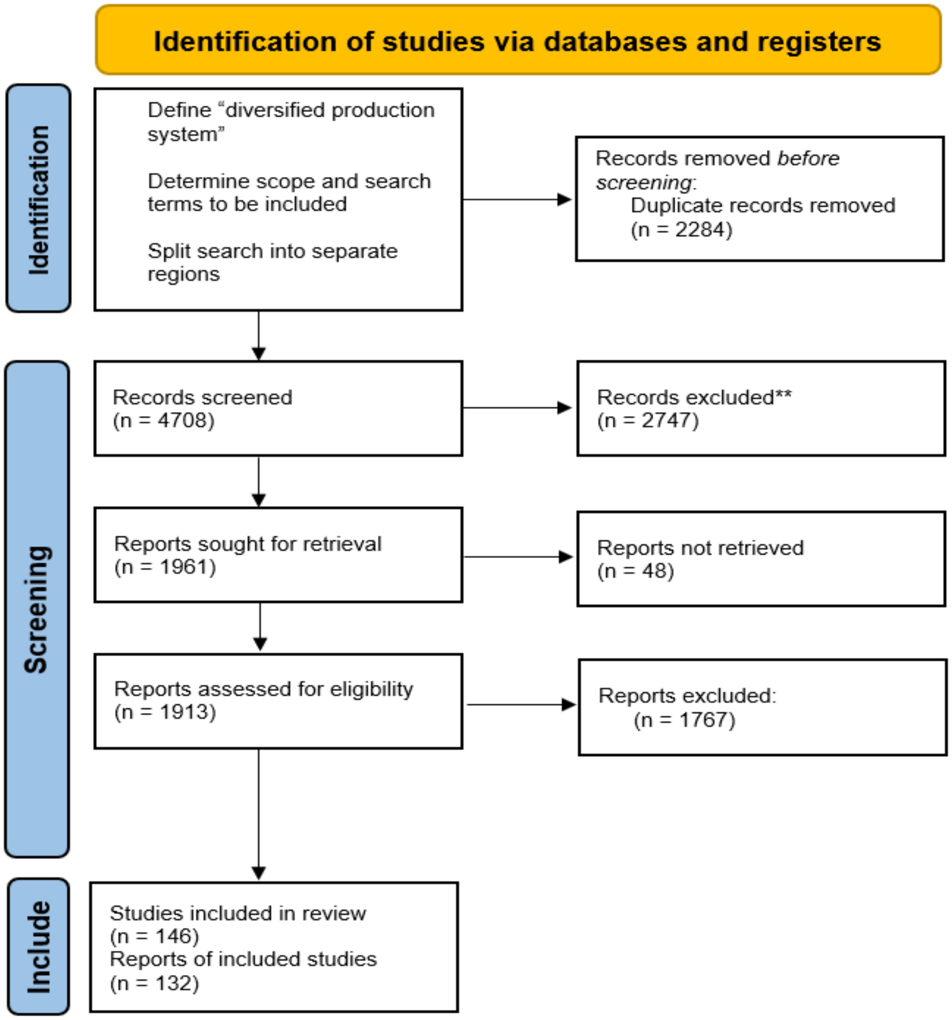

**Figure 3.2.** Literature review flowchart

*Table 3.1. Boolean combinations of agricultural management terms.*

| | |
|---|---|
| **Cropping Systems**<br>**AND**<br>**Soil Health** | TOPIC: (Cropland* OR "cropping system*" OR "agricultur* management" OR "regenerative agricultur*" OR "conservation agricultur*" OR "sustainable agricultur*" OR "soil management" OR "soil management practice*" OR agro-ecosystem* OR agroecosystem* OR crop OR "field crop*" OR farm* OR "conservation agricult*" OR "diversified farming system" OR "climate smart agricultur*" OR "climate-smart" OR "organic")<br>AND TOPIC: ("soil organic carbon" OR "SOC" OR SOM OR "soil organic matter") |

| | |
|---|---|
| **Cropping Diversification** | TOPIC: ("cover crop" OR "cover-crop*" OR "cover crop*" OR “green manure” OR “crop* rotation*” OR “mixed crop*” OR “diverse crop*” OR “crop* divers*” OR “crop* intensity” OR “sustainable intensification” OR rotation OR “double crop*” OR perennialization OR perennial* OR "perennial * crop*" OR “interseed”) |
| **Tillage Management** | TOPIC: ("conservation till*" OR "conventional till" OR "no-till" OR "no till*" OR "reduced till*" OR "minimum till*") |
| **Integrated Livestock** | TOPIC: ("integrated livestock" OR “livestock integration” OR “integrated crop-livestock” OR ” integrated crop-livestock system” OR “grazing inclusion” OR “crop grazing” OR “mixed crop-livestock” OR “mixed crop-livestock system” OR crop-livestock OR “integrated grazing”) |
| **Pest Management** | TOPIC: (pesticide seed treatment* OR "seed treatment*" OR "systemic insect*" OR neonic* OR pyrethr* OR (foliar AND insecticide*) OR “integrated pest management” OR IPM) |

### *3.2 Analytical approach*

We categorized studies and the regenerative practices examined by USDA’s four soil health principles as shown in Table 3.2.

*Table 3.2. Categorization of soil health principles and implementing practices.*

| **Soil health principle** | **Practices** |
|---|---|
| Maximize Presence of Living Roots | • Cover Crops<br>• Cropping System Intensification |
| Minimize Disturbance | • No till<br>• Amendment with Organic Nutrients (e.g., Compost, Manure)<br>• Organic Pest Control (e.g., Pollinator Strips) |

| | |
|---|---|
| Maximize Soil Cover | - Cover crops<br>- Cropping System Intensification (defined below) |
| Maximize Biodiversity | - Integration of Livestock[5]<br>- Cropping System Intensification<br>- Increased diversity of crop rotation |

Cropping system intensification refers to the number of crops grown in a single piece of ground over the course of one year and may include. i) eliminating summer fallow in monocrop systems, ii) increasing the number of annual crops grown per year, iii) including perennial crops in rotation. "Increased diversity of crop rotation" refers to increasing the diversity of crops grown by at least 2 species within a defined period of time, for instance changing from 2 crops grown over the course of two years to at least four.

### *3.3 Analysis of soil carbon stock changes in regenerative systems*

Studies reportedSOC impacts on soil carbon both in terms of changes to concentration and changes to soil carbon stocks. Since changes to soil carbon concentration are of limited value in determining climate change impacts, we limited our analysis of climate impacts to those studies reporting changes in SOC stocks. While measurements of carbon concentrations are useful for assessing overall impacts on soil health, they are not sufficient to assess climate change impacts because they cannot determine a specific amount of carbon in a particular area of soil. Instead, measurements of soil carbon stocks are needed, which account for both the concentration of carbon in the soil and the soil's bulk density, which measures the amount of soil per unit volume. Further, few studies contained baseline measurements of SOC stocks. Therefore, our analysis is restricted to reporting differences in SOC levels

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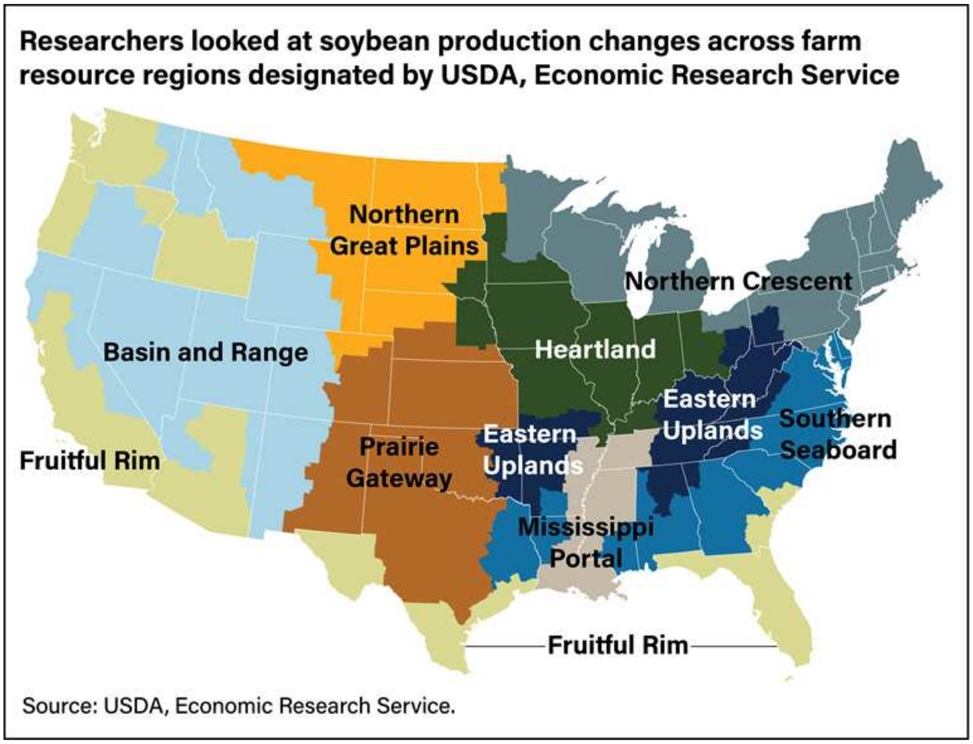
[5] Studies did not allow for assessment of the impacts of different grazing management systems (e.g., continuous grazing vs. rotational grazing).

and not the cause of those changes, for instance a slowing of SOC losses or an increase in plant inputs. While both have positive climate implications as compared to the control, the policy implications differ.

We analyzed the remaining studies based on 1) the time since practice implementation; 2) the number of practices and their combined effect; and 3) the regions in which studies were located. For all analyses, we used the SOC stocks measured to the deepest layer reported by the authors.

For the regional analysis, we used USDA Economic Research Services' Farm Resource Regions (Fig. 3.3) (Heimlich, 2000). This characterization has the benefit of accounting for important policy considerations, namely similar geomorphic, climatic, and soil characteristics, as well as the specialization of production types and commodities.



Researchers looked at soybean production changes across farm resource regions designated by USDA, Economic Research Service

Source: USDA, Economic Research Service.

**Figure 3.3.** Farm Resource Regions combine areas of analogous farm types with uniform physiographic, soil, and climatic characteristics as defined by USDA's Land Resource Regions and are aligned with the boundaries of NASS Crop Reporting Districts, which are aggregates of counties.

### *3.4 Soil Health and Yield Impacts*

Table 3.3 shows the soil health metrics examined and their importance. Unlike the climate analysis, studies measuring concentration, as well as those measuring stocks, were included. We recorded all soil health metrics in regenerative systems as either improved (1), unchanged (0), or declined (-1) compared to conventional management. When comparing yield between regenerative and conventional systems, we reported the difference in yield for the same cash crop, as presented by the authors.

*Table 3.3. Soil health indicators examined.*

| **Metric** | **Definition** | **Importance** |
|---|---|---|
| Respiration | Amount of microbial activity | Soil fertility |
| Active Carbon | Pool of SOC that can be easily decomposed or metabolized by microbial organisms. Includes dissolved organic carbon, particulate organic carbon, and permanganate oxidizable carbon | Soil fertility |
| Bacteria Diversity | Extent of different types of bacteria in the soils | Soil fertility, disease suppression, soil structure |
| Fungi Diversity | Extent of different types of bacteria in the soils | Soil fertility; potential for beneficial mycorrhizal associations; improvements to soil structure, disease suppression, overall biodiversity support |
| Fauna Diversity | Diversity of insects and other organisms (e.g., earthworms, beetles, protozoa, and nematodes) | Soil structure, nutrient and carbon cycling, soil fertility |

|  |  |  |
|---|---|---|
| Aggregate Stability | Ability of soil aggregate to resist erosion and other disruptive forces | Erosion resistance, water infiltration, root penetration, and microbial habitat |
| Water Holding Capacity | Ability of soil to retain and store water | Drought resilience |
| Potentially Mineralizable Nitrogen | Amount of nitrogen in the soils that is potentially plant available | Soil fertility |

## 4. FINDINGS

The results of this study indicate that regenerative agricultural systems have significantly more positive impacts compared to conventional controls. These benefits extend across SOC and climate impacts, biological and chemical indicators of soil health, and yield outcomes.

### *4.1 Impacts on Soil Organic Carbon*

Our findings strongly suggest that regenerative systems have more positive climate impacts than single-practice systems, with the length of practice implementation being the most influential factor. Across all 129 studies, 80% showed higher SOC stocks in regenerative agricultural systems as compared to a conventional control with differences ranging between -15.3 and 76.9 megagrams of SOC per hectare (Mg C $ha^{-1}$). In fact, 76% showed that regenerative systems had greater than one Mg C $ha^{-1}$ compared to conventional controls (Fig. 3.4). Many of the studies examined (n = 101) did not quantify SOC stocks and only reported SOC concentrations (Fig. 3.4)

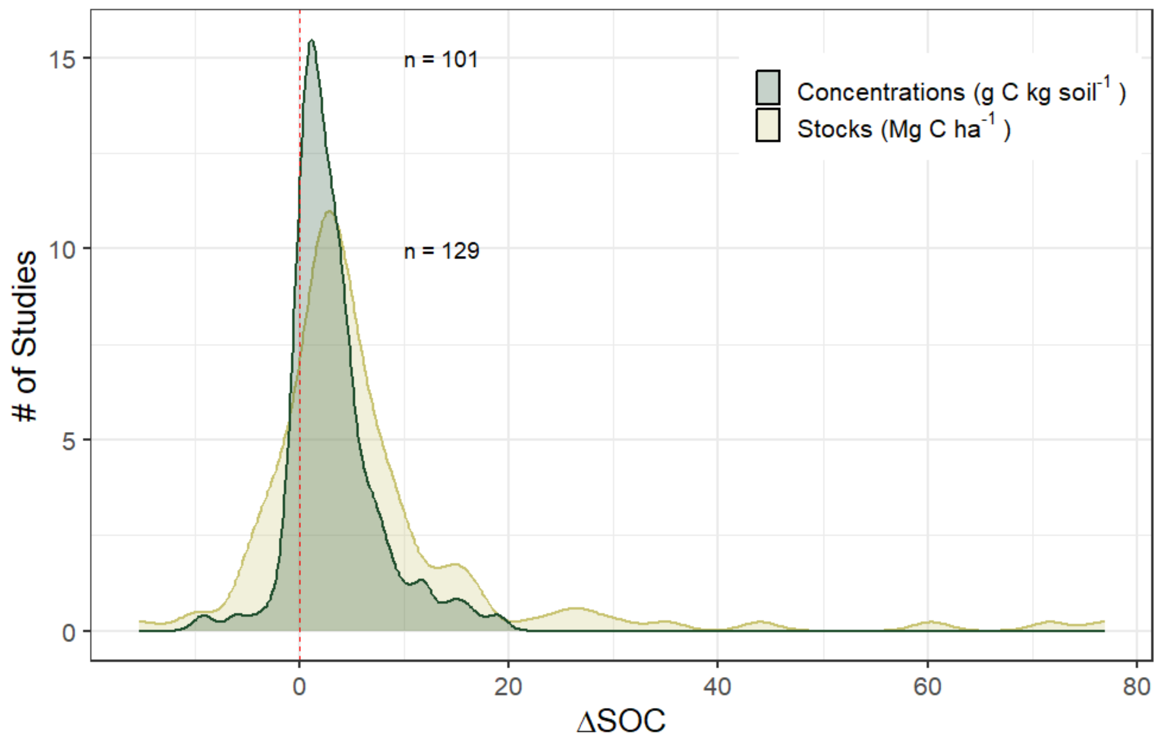


**Figure 3.4**. Frequency distribution of changes in SOC as reported in the literature. Of the 129 observations of stock changes, 123 studies that reported stock changes also reported concentration changes. 101 studies reported only SOC concentrations and not stocks.

### *4.2 Length of Practice Adoption*

Aside from climate, the largest factor affecting soil carbon stocks is the length of practice adoption with longer timeframes having more positive climate impacts. In studies less than 10 years, 76% of regenerative systems showed increased levels of SOC as compared to the conventional control with most studies reporting up to 5 tons per acre (Fig. 3.5). In studies of ten years or more, the effect is more pronounced with 94% of studies reporting positive changes in SOC stocks in regenerative agricultural systems (Fig. 3.5).

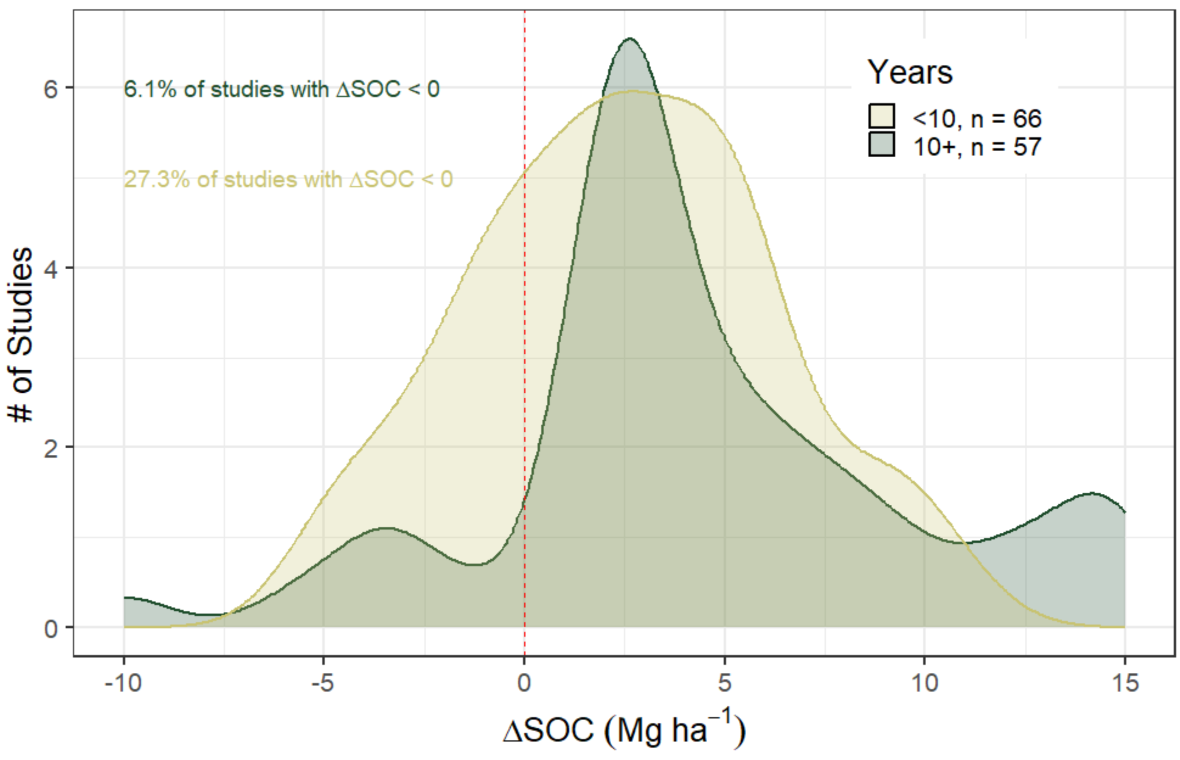


**Figure 3.5**. The frequency distribution of changes in soil organic carbon (SOC) stocks is shown as a function of study duration. Fifty-seven of the studies had durations of 10 years or more, while 66 studies had durations of less than 10 years.

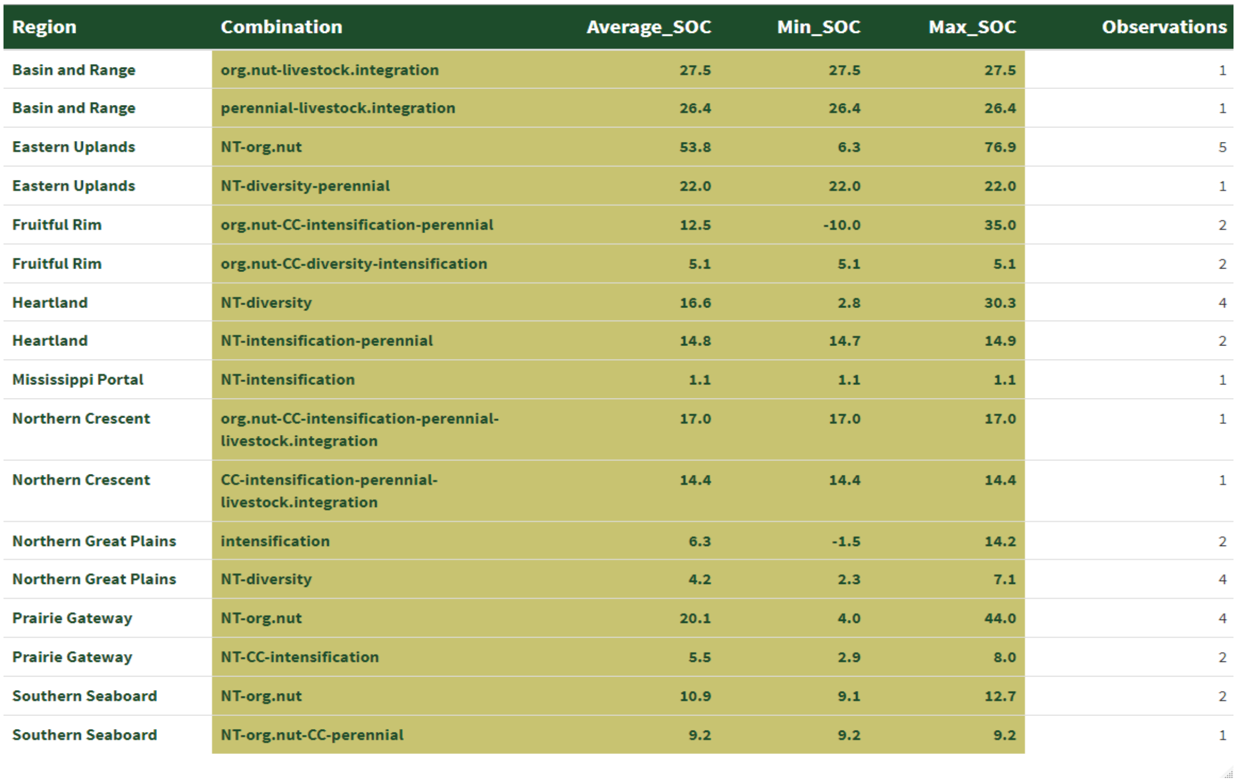
### *4.3 Combinations of practices*

Our findings strongly indicate that the efficacy of practices in maintaining or increasing carbon stocks varies by region but is greatly improved by stacking multiple practices together (Lehman et al., 2019; Prairie et al., 2023; Villat and Nicholas, 2024). Table 3.4 shows the top two combinations of practices in each ERS region, with all but one combination involving multiple practices. Although the reviewed studies covered a range of practice combinations, they did not evaluate all potential approaches, especially within a region. Therefore, while robust, these findings highlight the need for additional research.

No-till is the most commonly studied soil health practice (Paul et al., 2019). When combined with organic nutrients (e.g., compost or manure) or livestock integration, it showed the highest SOC stocks compared to conventional controls. Specifically, three studies in corn systems in the Eastern Uplands reported differences in carbon stocks of 60.3, 71.6, and 76.9 Mg C ha$^{-1}$ in the top 30cm of soil after 21, 15, and 21 years, respectively, compared to conventional controls (Table 3.4). These significantly higher impacts highlight the need for long-term studies and implementation of these systems. Although livestock integration has not been extensively studied (only 16 studies), it consistently emerged as one of the most influential practices when combined with others (Table 3.4).

These findings are unsurprising. First, numerous studies confirm that no till is critical to maintaining and building carbon stocks (Franzluebbers, 2010; Luo et al., 2010; Ogle et al., 2012). In a no till system, carbon can become protected in soil aggregates and become more resistant to loss through decomposition and erosion (Six et al., 1999; Devine et al., 2014). Second, manure and compost amendments add carbon directly to the system. Third, integrating livestock contributes carbon directly through manure deposition and can stimulate carbon exudation through plant roots (Brewer and Gaudin, 2020) which can enhance SOM stabilization (Piñeiro et al., 2009). Rasmussen et al. (2024) likewise found that livestock integration was the single most influential practice with respect to positive outcomes.

*Table 3.4. Table of the top combinations of agricultural practices contributing to soil organic carbon (SOC) stock change in megagrams per hectare (ΔSOC Mg ha$^{-1}$) compared to a conventional control without soil health practices. The regions are organized based on USDA's Farm Resource Region classification system.*



| Region | Combination | Average_SOC | Min_SOC | Max_SOC | Observations |
|---|---|---|---|---|---|
| Basin and Range | org.nut-livestock.integration | 27.5 | 27.5 | 27.5 | 1 |
| Basin and Range | perennial-livestock.integration | 26.4 | 26.4 | 26.4 | 1 |
| Eastern Uplands | NT-org.nut | 53.8 | 6.3 | 76.9 | 5 |
| Eastern Uplands | NT-diversity-perennial | 22.0 | 22.0 | 22.0 | 1 |
| Fruitful Rim | org.nut-CC-intensification-perennial | 12.5 | -10.0 | 35.0 | 2 |
| Fruitful Rim | org.nut-CC-diversity-intensification | 5.1 | 5.1 | 5.1 | 2 |
| Heartland | NT-diversity | 16.6 | 2.8 | 30.3 | 4 |
| Heartland | NT-intensification-perennial | 14.8 | 14.7 | 14.9 | 2 |
| Mississippi Portal | NT-intensification | 1.1 | 1.1 | 1.1 | 1 |
| Northern Crescent | org.nut-CC-intensification-perennial-livestock.integration | 17.0 | 17.0 | 17.0 | 1 |
| Northern Crescent | CC-intensification-perennial-livestock.integration | 14.4 | 14.4 | 14.4 | 1 |
| Northern Great Plains | intensification | 6.3 | -1.5 | 14.2 | 2 |
| Northern Great Plains | NT-diversity | 4.2 | 2.3 | 7.1 | 4 |
| Prairie Gateway | NT-org.nut | 20.1 | 4.0 | 44.0 | 4 |
| Prairie Gateway | NT-CC-intensification | 5.5 | 2.9 | 8.0 | 2 |
| Southern Seaboard | NT-org.nut | 10.9 | 9.1 | 12.7 | 2 |
| Southern Seaboard | NT-org.nut-CC-perennial | 9.2 | 9.2 | 9.2 | 1 |

## *4.4 Practices by region*

Figure 3.6 shows the impact of different soil health practices on carbon stocks by USDA Farm Resource Region. Pie chart sizes are proportional to the number of observations in each region divided by the total number of observations of the dataset. Each pie chart's wedge sizes represent the average SOC stock difference attributable to specific agricultural practices as a fraction of the total regional average SOC stock difference. For instance, The Basin and Range region is relatively unstudied, with the literature addressing only six practices, each having a roughly equivalent effect on carbon stocks. In contrast, the Heartland region, representing much of the Midwest, is well-studied in both the number of studies and practices examined.

These findings demonstrate that the impact of practices on SOC stocks is highly context-dependent. Livestock integration explained the most variation in the Heartland and Northern Crescent. Organic nutrient addition was most impactful in the Prairie Gateway, and cover crops had the greatest impact in the Northern Great Plains. In the Fruitful Rim, perennial systems were the most effective. In the

Basin and Range, no-till, livestock integration, perennial systems, and diversity had roughly the same impacts.

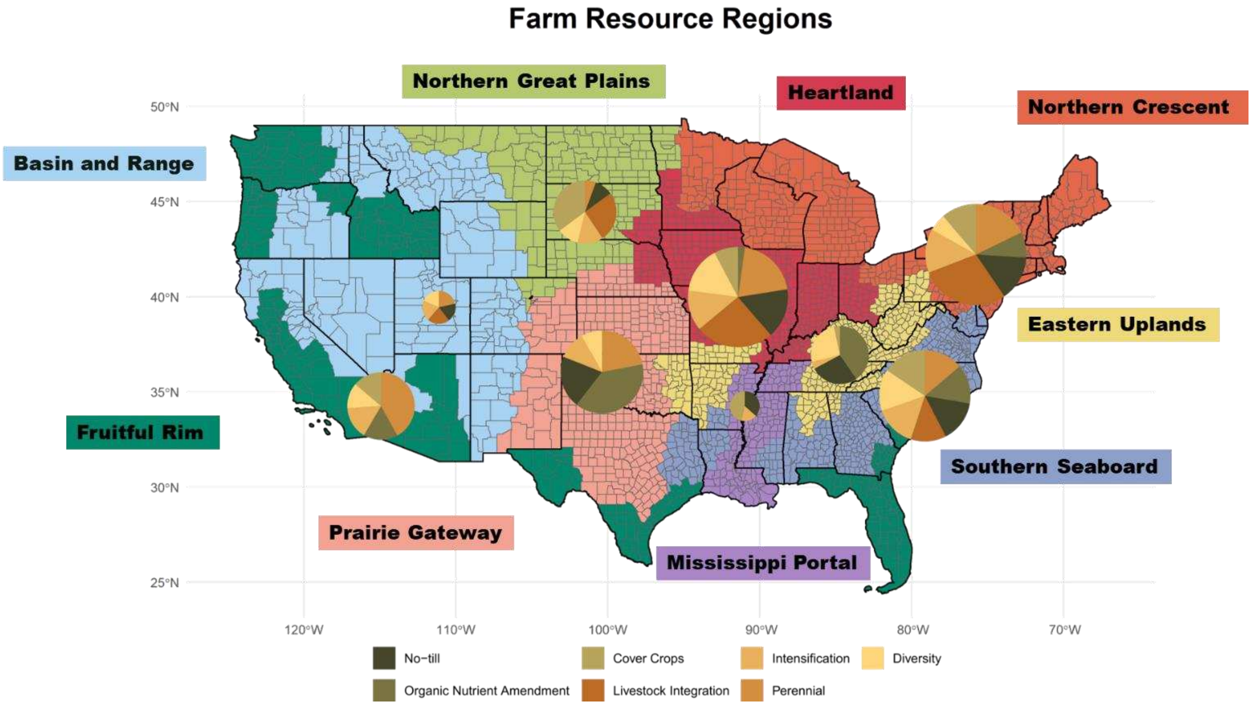

Farm Resource Regions

**Figure 3.6**. The map presents a visualization of the relative impact of regenerative practices on soil organic carbon stock (SOC) changes across the USDA Farm Resource Regions. Pie chart sizes are proportional to the number of observations in each region divided by the total number of observations in the dataset. Each pie chart's wedge sizes represent the average SOC stock change attributable to specific agricultural practices as a fraction of the total regional average SOC stock change.

As with other findings, more research is needed to increase our confidence in these results. Our findings are also limited by the practice combinations actually studied in these regions. Only the Heartland, Northern Crescent, and Southern Seaboard had studies testing all seven practices. Limitations in the Basin & Range and Mississippi Portal areas are particularly concerning, as these regions had significantly fewer studies—six and four studies, respectively. Additionally, the Basin and Range tested five of the seven practices, while the Mississippi Portal tested only three of the seven.

### *4.5 Carbon at depth*

As discussed above, soil C changes can occur in both surface layers and deeper layers of the soil and understanding changes in both are critical. Of the 129 studies examined, 74% examined changes only in topsoil data[6] with the remaining studies (33) measured down to the subsoil (Fig. 3.7).[7] Accounting for subsoil SOC is crucial, as 66% of studies report SOC increases when including subsoil measurements, compared to 85% for topsoil only. Additionally, the maximum observed SOC loss reduces from -15.2 Mg C ha$^{-1}$ to -5.6 Mg C ha$^{-1}$ when subsoil is considered. Including subsoil measurements could alter the perceived climate change impacts of regenerative farming systems (Knebl et al., 2017).

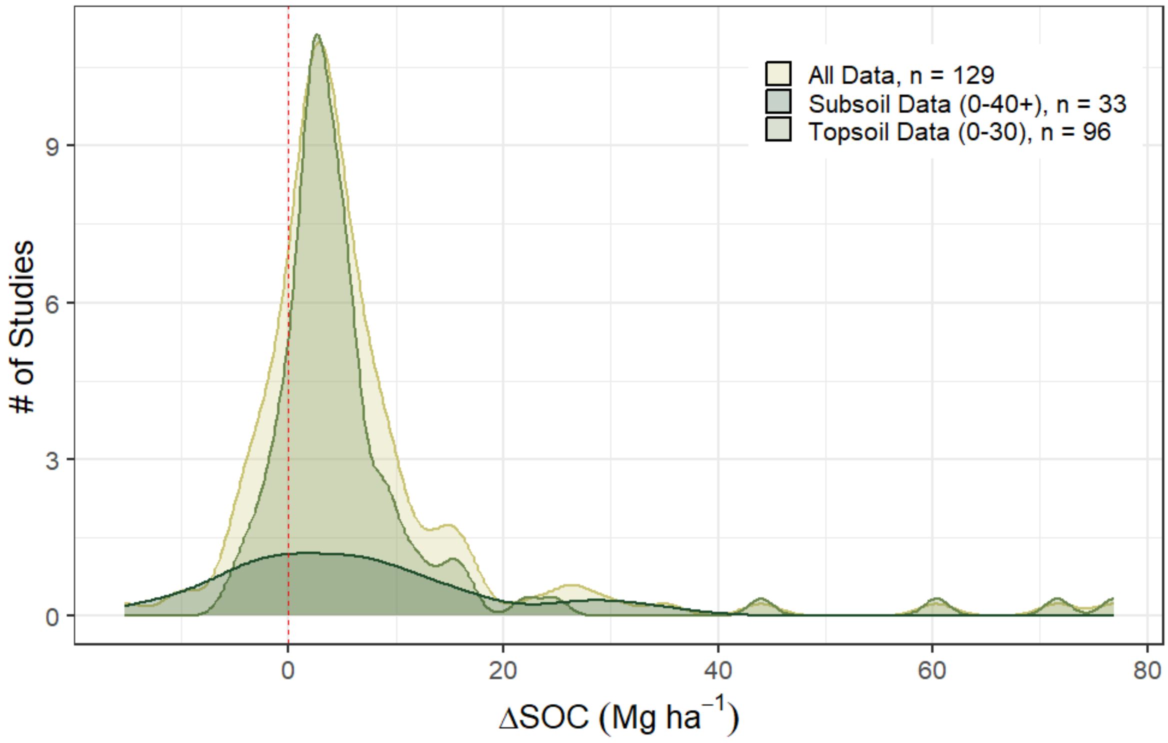


**Figure 3.7.** Changes in SOC stocks as reported in the literature. Ninety-six observations were for topsoil and 33 for subsoil, contributing to their respective distributions out of a total of 129 observations.

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[6] Topsoil data refers to measurements from 0 to 30 cm (11.8 inches).

[7] Subsoil data refers to measurements from 0 to greater than 40 cm (15.5 inches).

### *4.6 Higher yields and healthier soils with regenerative agricultural systems*

Our research shows that on the whole, regenerative agricultural systems support greater soil health and higher yields than conventional controls (Figure 3.8). While some studies showed either a decrease (-1) or no change (0) on biological and chemical parameters and yield, the vast majority of papers showed positive effects. The effects on yield are notable, suggesting that regenerative systems are feasible from an economic perspective. Furthermore, soil health metrics related to drought resilience (water holding capacity), nutrient cycling (potentially mineralizable nitrogen, PMN), resistance to erosion (aggregate stability), and biodiversity all showed significant increases in regenerative agricultural systems, highlighting the additional benefits of adopting these practices.

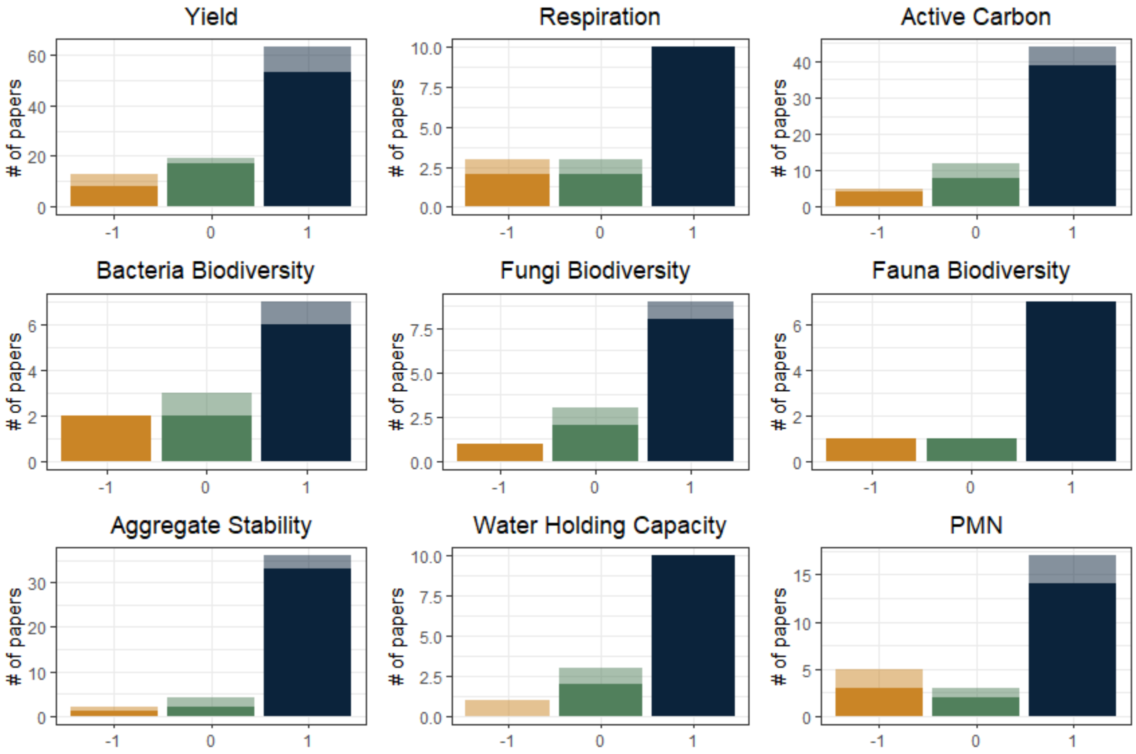

**Figure 3.8**. The impact of regenerative management on yield and soil health indices. The x-axis categories indicate the direction of change: a decrease (−1), no change (0), and an increase (+1) in the parameter in response to SOC changes. Transparent regions on the bars indicate studies with a decrease in soil organic carbon (SOC) stocks (ΔSOC < 0), while the darker shade represent studies with an increase in SOC stock (ΔSOC > 0). Active carbon refers dissolved organic carbon (DOC), particulate organic carbon (POC), and permanganate oxidizable carbon (POXC). PMN refers to potentially mineralizable nitrogen.

## 5. RECOMMENDATIONS

Our research points to two main sets of suggestions to support increased transition by farmers to regenerative agricultural systems: 1) Adjustments to USDA NRCS programs to optimize the positive carbon and soil health impacts and 2) funding for research and other activities that will fill critical information gaps.

### *5.1 Increase funding for carbon positive soil health practices under the Conservation Stewardship Program and the Environmental Quality Incentives Program*

The Conservation Stewardship Program (CSP) provides assistance to farmers and ranchers seeking to maintain and adopt a suite of conservation practices on their operations. While EQIP takes an a la carte approach that is helpful for producers who are new to adopting soil health practices. Despite being the most significant conservation program, funding for CSP has been steadily declining and only about ¼ of applicants receive CSP contracts (Coppess, 2023). In contrast, funding for EQIP has steadily increased, but it too remains oversubscribed with approximately just one quarter of applicants receiving contracts ("Environmental Quality Incentives Program," 2024).

CSP and EQIP provide funding for the adoption of several types of agricultural management practices, not all of which directly support positive soil carbon and soil health outcomes. Given the critical importance of soil health and soil organic carbon in the face of accelerating climate risks, *Congress should provide more funding for and place increasing programmatic emphasis on carbon positive soil health practices in a manner that does not reduce funding for other important conservation practices.*

### *5.2 Support longer participation times in CSP, EQIP, and other federal programs*

As our findings demonstrate, more positive carbon benefits are seen at a point of ten years or more and ample experience confirms that soil health benefits may take up to five to seven years to accrue (Prairie et al., 2023). Congress should therefore incentivize longer participation times in all USDA NRCS programs that promote soil health or positive SOC outcomes. Specifically, *Congress should reinstate the automatic renewal provision for CSP contracts so that farmers who have completed five years may participate for up to ten years. Further, Congress should consider whether certain circumstances warrant contracts of more than ten years.*

### *5.3 Incentivize regionally effective practices and encourage a combination of practices*

As Table 3.4 and Figure 3.6 demonstrate, the efficacy of practices in creating positive carbon impacts varies by region, but is greatly improved when employing a diverse combination of practices. Therefore, we recommend that conservation professionals consider this data when developing rankings for practices.

### *5.4 Provide resources and fund research to fill critical information gaps*

As emphasized above, significant gaps in existing research limit the application of our findings. This section outlines several recommendations for both research protocols, areas of study, and agency action to fill these gaps.

#### *5.4.1 Institute rigorous research protocols*

Federally supported research and programmatic monitoring should have the following to ensure scientific rigor:

- Valid controls;
- Baseline measurements of all relevant metrics, including SOC and soil health metrics;
- Measurements of soil organic carbon stocks down to at least 30 cm and ideally up to a meter or more to capture the entire soil profile;
- Measurements of other GHGs from soils, such as soil $N_2O$ emissions, to facilitate a more complete understanding of the GHG profiles of operations; and
- Intermediate measurements soil health metrics and SOC to determine the rate of impacts.
- Where possible we recommend that studies be long-term, for a period of ten years or more.

Additionally, rather than simply recommending more extensive sampling, which can be prohibitively expensive, we propose setting up regional long-term research farms. These farms would serve as representative sites for their respective regions, providing valuable baseline data and long-term datasets.

This approach allows for the validation of regionally appropriate models and the prediction of regional trends, thus reducing overall sampling costs. By focusing research on these representative farms, we can generalize findings to broader areas within each region effectively. This regional aspect of the study enhances its novelty and applicability, offering a scalable solution to the challenge of comprehensive sampling.

### *5.4.2 Expand the types of crops and practices studied*

Predictably, research efforts have predominantly targeted a select few commodity crop systems, such as corn, soy, wheat, and cotton. This focus is understandable given that commodity crops receive the majority of Farm Bill funding and have the most significant economic impact on the agricultural sector. (Johnson and Monke, 2024) Additionally, the studied conservation practices have mainly centered on well-established techniques already eligible for payments under NRCS conservation programs. However, this narrow focus has created a self-reinforcing cycle that excludes alternative production systems and innovative soil conservation methods. This limitation must be overcome to ensure that policies support effective responses to climate change. First, the efficacy of many frontier practices, including biochar, agroforestry, and inoculants, may match if not supersede that of existing practices both in terms of carbon impacts but also soil health and yield outcomes (Muchane et al., 2020; Alkharabsheh et al., 2021). Second, responding to climate change will likely require relying on regenerative crops both for purposes of increasing diversity in rotations and for consumption, including drought resilient crops such as millet.

### *5.4.3 Emphasize integrated crop-livestock systems*

Despite some limitations in research, studies on livestock integration consistently demonstrate significant positive impacts on soil organic carbon (SOC) stocks across various regions, including the Northern Great Plains, the Heartland, and the Northern Crescent (Fig. 3.6). The combination of livestock integration with other soil health practices—such as cover cropping, intensification, and the use of perennials—has shown substantial benefits for SOC in these areas (Table 3.4). These practices have the potential to be synergistic, building more SOC than single practices alone (Prairie et al. 2023, Rasmussen et al. 2024). Moreover, grazing livestock on cover crops provides an additional revenue stream, turning what would otherwise be a sunk cost into income. Research into crop-livestock systems should focus on the comparison of specific management strategies such as high-density, rotational grazing compared to low-density, continuous grazing.

### *5.4.4 Fill critical gaps by region*

As Figure 3.6 demonstrates, the vast majority of studies were conducted in the Heartland, Northern Crescent, and Southern Seaboard. This geographical focus aligns with the emphasis on commodity crops. However, this leaves important areas of the country understudied, notably the Basin and Range area. While this region has relatively little cropland, understanding the efficacy of practices here is critical due to its potential for climate mitigation in vast rangelands and its importance to US grain production.

### *5.4.5 Expand USDA reporting of soil health practices*

Following the above recommendation, Congress should require the USDA to gather and report detailed data on soil health practices through the Census of Agriculture. Currently, the Census reports primarily on the use of no-till, reduced till, intensive tillage, and cover crops ("List of Reports and Publications | 2022 Census of Agriculture | USDA/NASS," 2022). It also reports on amendment applications but lacks detail to determine whether these amendments support soil health or positive carbon outcomes. Additionally, while the Census reports the amount of organic farming, it does not provide details on how these operations are managed.

Producers, whether on organic or conventional operations, implement a variety of other practices that should be captured by the Census of Agriculture. This data would provide valuable information on the prevalence of practices within regions and nationally, assisting researchers and the USDA in determining which practices merit further study and/or federal support.

### *5.5 Research limitations*

In general, our findings confirm that regenerative agricultural systems support increased SOC stocks and greater soil health and yields than conventional systems. As noted above, however, our findings are subject to certain limitations. First, the climate implications of our findings are limited to assessing the relative carbon stocks in systems with different treatments. Since qualifying studies did not contain baseline soil measurements, we cannot attribute differences to carbon sequestration as opposed to a slower loss in carbon stocks. Moreover, this study does not account for differences in $N_2O$ emissions between regenerative and conventional systems, which could impact the directionality and/or magnitude of the climate impact. At the outset, it is important to note that despite the strength of these findings, our findings cannot be interpreted to extend to carbon sequestration for the reasons explained above, namely: 1) lack of baseline measurements in many studies and 2) a lack of intermediate sampling during the study period. For these reasons, we were unable to quantify rates of soil carbon sequestration, only differences in SOC stocks between treatments at a single point in time.

Second, regarding the regional importance of different practice combinations are limited by both the number of studies and the practices examined. In general, studies were conducted only on a few types of production systems - namely, corn, soy, wheat, and cotton. Finally, the practices examined were quite limited and did not include frontier practices, such as microbial inoculants, rock dust amendments, agroforestry, biochar, and foliar amendments, which may result in more carbon sequestration and greater soil health benefits. As discussed below, it is critically important to increase not only the number of studies, but also the diversity of production types and practices.

Third, the response of SOC to regenerative management at depths greater than 30cm is critical for evaluating the soil C sequestration potential of regenerative agriculture.

Most studies did not permit estimating carbon impacts of practices because they did not measure C stocks in the soil. As noted above, two different methods exist for measuring carbon in soils: 1) measuring the concentration of carbon and 2) measuring carbon stocks within a given area. While measuring carbon concentrations is important for understanding impacts of practices on soil health metrics, measuring C stocks is necessary to estimate carbon sequestration changes.

Measuring C stocks requires measuring both carbon concentrations and the bulk density of the soils.[8] Forty-four percent of the studies that we examined were excluded from SOC analysis because they did not measure stocks.

## 6. CONCLUSION

Our findings are consistent with a growing body of literature demonstrating that regenerative agricultural systems are associated with a range of beneficial outcomes. These systems not only improve soil health, yield, and environmental conditions but also positively impact social outcomes, such as human well-being (Rasmussen et al., 2024). Regenerative systems enhance SOC stocks, improve drought resilience, nutrient cycling, and resistance to erosion, and increase biodiversity.

These practices contribute to more stable and productive agricultural ecosystems capable of withstanding climatic fluctuations and other stressors. Our research emphasizes the importance of implementing multiple soil health practices to achieve these benefits, as combining practices often results in more significant improvements than single practices alone.

This study highlights the critical need for practical analyses to inform policy, emphasizing a less scientifically rigorous but broader impact approach. By providing a comprehensive review of existing research on regenerative agricultural systems, we aim to support policymakers in making informed decisions that promote sustainable and resilient agricultural practices. The positive impacts of regenerative systems on SOC and other soil health metrics align with global efforts to mitigate climate change and promote sustainable land management.

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[8] Bulk density is the mass of soil per unit of volume usually measured in g $cm^{-3}$

Further research is needed to fill existing gaps, particularly in understudied regions and with less-explored practices. Long-term studies and detailed measurements of SOC stocks at various soil depths are crucial for accurately assessing the climate mitigation potential of regenerative systems. Additionally, expanding research to include a wider variety of crops and innovative soil health practices will provide a more comprehensive understanding of the benefits and challenges associated with these systems.

In conclusion, our findings support the integration of regenerative agricultural systems as a viable strategy for enhancing soil health, improving yields, and contributing to broader environmental and social goals. By adopting these practices, farmers can build more resilient agricultural landscapes, contributing to the overall sustainability and productivity of the agricultural sector.

## REFERENCES

Alkharabsheh, H., Seleiman, M., Battaglia, M., Shami, A., Jalal, R., Alhammad, B., Almutairi, K., Al-Saif, A., 2021. Biochar and Its broad impacts in soil quality and fertility, nutrient leaching and crop productivity: A review. AGRONOMY-BASEL 11. doi:10.3390/agronomy11050993

Bowman, M., Poley, K., McFarland, E., 2022. Farmers employ diverse cover crop management strategies to meet soil health goals. Agricultural & Environmental Letters 7, e20070. doi:10.1002/ael2.20070

Brewer, K.M., Gaudin, A.C.M., 2020. Potential of crop-livestock integration to enhance carbon sequestration and agroecosystem functioning in semi-arid croplands. Soil Biology and Biochemistry 149, 107936. doi:10.1016/j.soilbio.2020.107936

Coppess, J., 2023. The incredible shrinking of the conservation stewardship program. Farmdoc Daily 13.

Devine, S., Markewitz, D., Hendrix, P., Coleman, D., 2014. Soil Aggregates and associated organic matter under conventional tillage, no-Tillage, and forest succession after three decades. PLOS ONE 9, e84988. doi:10.1371/journal.pone.0084988

Environmental Quality Incentives Program [WWW Document], 2024. . National Sustainable Agriculture Coalition. URL https://sustainableagriculture.net/publications/grassrootsguide/conservation-environment/environmental-quality-incentives-program/ (accessed 6.6.24).

Fenster, T., Oikawa, P., Lundgren, J., 2021. Regenerative almond production systems improve soil health, biodiversity, and profit. Frontiers in Sustainable Food Systems 5. doi:10.3389/fsufs.2021.664359

Franzluebbers, A.J., 2010. Achieving soil organic carbon sequestration with conservation agricultural systems in the southeastern United States. Soil Science Society of America Journal 74, 347–357. doi:10.2136/sssaj2009.0079

Heimlich, R., n.d. Farm Resource Regions [WWW Document]. URL http://www.ers.usda.gov/publications/pub-details/?pubid=42299 (accessed 5.30.24).

Johnson, R., Monke, J., 2024. Farm bill primer: What Is the farm bill?

Knebl, L., Leithold, G., Schulz, F., Brock, C., 2017. The role of soil depth in the evaluation of management-induced effects on soil organic matter. European Journal of Soil Science 68, 979–987. doi:10.1111/ejss.12492

Lehman, R.M., Osborne, S.L., McGraw, K., 2019. Stacking agricultural management tactics to promote improvements in soil structure and microbial activities. Agronomy 9, 539. doi:10.3390/agronomy9090539

List of Reports and Publications | 2022 Census of Agriculture | USDA/NASS [WWW Document], 2022. URL https://www.nass.usda.gov/Publications/AgCensus/2022/index.php (accessed 6.6.24).

Luo, Z., Wang, E., Sun, O.J., 2010. Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. Agriculture, Ecosystems & Environment 139, 224–231. doi:10.1016/j.agee.2010.08.006

Tahat, M., M. Alananbeh, K., A. Othman, Y., I. Leskovar, D., 2020. Soil health and sustainable agriculture. Sustainability 12, 4859. doi:10.3390/su12124859

Muchane, M.N., Sileshi, G.W., Gripenberg, S., Jonsson, M., Pumariño, L., Barrios, E., 2020. Agroforestry boosts soil health in the humid and sub-humid tropics: A meta-analysis. Agriculture, Ecosystems & Environment 295, 106899. doi:10.1016/j.agee.2020.106899

Ogle, S.M., Swan, A., Paustian, K., 2012. No-till management impacts on crop productivity, carbon input and soil carbon sequestration. Agriculture, Ecosystems & Environment 149, 37–49. doi:10.1016/j.agee.2011.12.010

Paul, E.A., Elliott, E.T., Paustian, K., Cole, C.V. (Eds.), 2019. Soil Organic Matter in Temperate Agroecosystems: Long-Term Experiments in North America. CRC Press, Boca Raton. doi:10.1201/9780367811693

Piñeiro, G., Paruelo, J.M., Jobbágy, E.G., Jackson, R.B., Oesterheld, M., 2009. Grazing effects on belowground C and N stocks along a network of cattle exclosures in temperate and subtropical grasslands of South America. Global Biogeochemical Cycles 23. doi:10.1029/2007GB003168

Prairie, A.M., King, A.E., Cotrufo, M.F., 2023. Restoring particulate and mineral-associated organic carbon through regenerative agriculture. Proceedings of the National Academy of Sciences 120, e2217481120. doi:10.1073/pnas.2217481120

Rasmussen, L.V., Grass, I., Mehrabi, Z., Smith, O.M., Bezner-Kerr, R., Blesh, J., Garibaldi, L.A., Isaac, M.E., Kennedy, C.M., Wittman, H., Batáry, P., Buchori, D., Cerda, R., Chará, J., Crowder, D.W., Darras, K., DeMaster, K., Garcia, K., Gómez, M., Gonthier, D., Guzman, A., Hidayat, P., Hipólito, J., Hirons, M., Hoey, L., James, D., John, I., Jones, A.D., Karp, D.S., Kebede, Y., Kerr, C.B., Klassen, S., Kotowska, M., Kreft, H., Llanque, R., Levers, C., Lizcano, D.J., Lu, A., Madsen, S., Marques, R.N., Martins, P.B., Melo, A., Nyantakyi-Frimpong, H., Olimpi, E.M., Owen, J.P., Pantevez, H., Qaim, M., Redlich, S., Scherber, C., Sciligo, A.R., Snapp, S., Snyder, W.E., Steffan-Dewenter, I., Stratton, A.E., Taylor, J.M., Tscharntke, T., Valencia, V., Vogel, C., Kremen, C., 2024. Joint environmental and social benefits from diversified agriculture. Science 384, 87–93. doi:10.1126/science.adj1914

Six, J., Elliott, E.T., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Science Society of America Journal 63, 1350–1358. doi:10.2136/sssaj1999.6351350x

Tully, K.L., McAskill, C., 2020. Promoting soil health in organically managed systems: a review. Organic Agriculture 10, 339–358. doi:10.1007/s13165-019-00275-1

Villat, J., Nicholas, K.A., 2024. Quantifying soil carbon sequestration from regenerative agricultural practices in crops and vineyards. Frontiers in Sustainable Food Systems 7. doi:10.3389/fsufs.2023.1234108

Workbook: RCA DV Erosion by State NRI 2017. URL https://publicdashboards.dl.usda.gov/t/FPAC_PUB/views/RCADVErosionbyStateNRI20171/ErosionTrends?%3Adisplay_count=n&%3Aembed=y&%3AisGuestRedirectFromVizportal=y&%3Aorigin=viz_share_link&%3AshowAppBanner=false&%3AshowVizHome=n (accessed 5.30.24).

Yang, T., Siddique, K.H.M., Liu, K., 2020. Cropping systems in agriculture and their impact on soil health-A review. Global Ecology and Conservation 23, e01118. doi:10.1016/j.gecco.2020.e01118

# CHAPTER 4: INTEGRATING REGENERATIVE AGRICULTURE: EFFECTS ON SOIL FAUNA BIODIVERSITY AND SOIL ORGANIC MATTER DYNAMICS[9]

## 1. INTRODUCTION

Soils are critical for delivering a multitude of ecosystem services, but their ability to do so depends on soil health. Healthy agricultural soils are those that sustain productivity by supporting nutrient recycling, water retention, and increased organic matter content (Smith et al., 2015). Our ability to feed a growing population relies on healthy soils that can sustain crop production in the face of challenges posed by climate change and degradation from extractive agriculture (FAO, 2020). Agricultural landscapes encompass a significant proportion of the world's soil, yet management practices vary widely and can either enhance or degrade soil health (Doran et al., 2002; Swift et al., 2004).

There is now widespread interest in regenerating soil health and reversing long-term trends of soil degradation. However, the adoption of regenerative practices remains low, particularly in major corn and wheat-producing states, partly due to a lack of understanding of how systems-level agricultural management affects soil health. Regenerative practices such as no-till, diversified cropping rotations, reduced synthetic inputs, cover crops, and livestock integration have been shown to improve soil health and biodiversity (Doran and Zeiss, 2000; Brussaard et al., 2007; Lemaire et al., 2014; McDaniel et al., 2014; Lehman et al., 2015; Liu et al., 2018; Rosenzweig et al., 2018; Fenster et al., 2021; Bowman et al., 2022). However, these practices are rarely considered together at a system level, and the challenge remains to elucidate their potentially synergistic impact on soil health.

Soil is a dynamic, living body containing a network of organisms operating through complex interactions with the abiotic environment (Brussaard, 1997). The capacity to manage soil health depends

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[9] Manuscript in preparation for peer review publication with Steven Rosenzweig, Jonathan Lundgren, Daniel Kane, and M. Francesca Cotrufo

on a mechanistic understanding of the biological processes provided by these organisms (Lavelle, 1997; Altieri, 1999; Kibblewhite et al., 2008; Parker, 2010) and how they respond to management. Soil fauna are essential for regulating nutrient flux, SOM dynamics, soil physical structure, and suppression of soilborne pests (Altieri, 1999; Lavelle et al., 2006, 2020; Barrios, 2007; Brussaard et al., 2007; Smith et al., 2015). These services constitute an important measure for the sustainable management of agricultural systems (Doran et al., 2002). Certain management practices can greatly influence the structure of these belowground communities and, subsequently, the functions they provide (Doran and Zeiss, 2000; Kibblewhite et al., 2008; Lehman et al., 2015). While soil health is believed to embrace the biological component of soil (Lehman et al., 2015), to date the focus has been on microbial diversity and functions (Córdova-Kreylos et al., 2006; McDaniel et al., 2014; Hartmann et al., 2015; Venter et al., 2016; Lori et al., 2017; Morugán-Coronado et al., 2022; Narayana et al., 2022, 2022; Paudel et al., 2023), and quantitative assessments of different regenerative practices on soil fauna abundance and diversity remains scarce (Puissant et al., 2021; Betancur-Corredor et al., 2022).

The most used soil health indicator throughout the world is soil organic matter (SOM), because of the numerous chemical, physical, and biological processes it influences (Lehman et al., 2015; Meena et al., 2024). However, SOM is incredibly complex and requires separation into multiple components with contrasting behavior to study and predict its dynamics. Separating SOM into particulate organic matter (POM) and mineral-associated organic matter (MAOM) offers a promising strategy to understand and predict broad-scale SOM dynamics due to the distinct formation pathways, vulnerability to disturbance and set of controls of these two pools (Lavallee et al., 2020; Cotrufo and Lavallee, 2022; Hansen et al., 2024, p. 202). POM cycles faster than MAOM and is considered a key indicator of soil fertility; however, POM is also more vulnerable to disturbance (Poeplau et al., 2018). Conversely, MAOM represents a SOM pool with, on average, slower turnover and can be used to assess management impacts on SOM stabilization and sequestration of atmospheric $CO_2$ (Hassink, 1997; Cotrufo et al., 2019).

Each of these pools of SOM has been studied from somewhat different perspectives, but there is a growing awareness that effective SOM management relies on a comprehensive understanding of how both POM and MAOM respond to management practices (Cotrufo and Lavallee, 2022; Angst et al., 2023). Particulate organic matter is formed from the fragmentation of plant and microbial structural inputs and can be physically occluded in aggregates, but it receives minimal protection from the soil matrix. Mineral-associated organic matter, is protected by soil mineral surfaces, leading to a finite storage capacity known as 'saturation' (Hassink and Whitmore, 1997; Cotrufo et al., 2013; Feng et al., 2013). The concept of C saturation in MAOM assumes a soil-specific upper limit, which has been widely adopted to estimate MAOM-C storage capacity both regionally and globally (Beare et al., 2014; Georgiou et al., 2022). Agricultural lands, particularly in the Southern Great Plains, have lost significant amounts of soil carbon and are far from saturation (Sanderman et al., 2017). This suggests that regenerative management efforts should focus on soils that are more depleted in SOC relative to their C saturation to maximize C storage in MAOM (Angst et al., 2023; Castellano et al., 2015).

To understand how soil fauna diversity, POM, and MAOM respond to management, it is crucial to study on-farm systems that employ multiple regenerative practices in combination. On farms, regenerative practices are often used in combination and stacking multiple soil health promoting practices is likely to have a greater beneficial impact on soil biodiversity and POM and MAOM dynamics than single practices (Nunes et al., 2018; Lehman et al., 2019; Prairie et al., 2023; Villat and Nicholas, 2024). However, the linkages between regenerative management, soil fauna diversity, and POM and MAOM remain unclear, especially within real on-farm systems.

Successful management for sustainable soil health must be implemented at the ecosystem level, integrating soil biological processes with human decision-making. Bridging this gap requires initiatives that catalyze farmer transitions to regenerative agricultural systems. One such pilot initiative launched by General Mills (GMI) in 2020 established educational and technical assistance programs with Understanding Ag to support farmers in developing and implement a regenerative agriculture management plan while receiving financial incentives delivered by GMI through the Ecosystem Services Market Consortium (ESMC) (GMI-ESMC-SGP). We selected 22 of these farms, which represented a broad range of historical management from over 100 years of conventional wheat farming to more regenerative systems with at least 10 years of no-till, cover crops, diverse cash crops, or integrated livestock. Our goal was to understand the relationship between management practices, soil biodiversity, and C and N storage in bulk SOM, as well as their distribution in POM and MAOM. To address the gaps in understanding and to provide a comprehensive evaluation of stacked regenerative agricultural practices effects on key soil health metrics, this study had the following objectives:

1) **Develop regenerative farming index (RFI)**: Create a continuous regenerative management variable through a scoring index by integrating normalized management scores for disturbance (tillage), cropping system intensity, crop diversity, cover crops, and livestock integration.
2) **Evaluate management effects on soil biodiversity and SOM dynamics**: Investigate the relationships between regenerative farming, soil fauna biodiversity, C and N stocks in SOM, POM and MAOM, and the edaphic controls of these relationships.

By achieving these objectives, this study aims to advance our understanding of how regenerative agricultural practices influence soil biodiversity, soil C sequestration, and SOM dynamics. This knowledge will be crucial for developing effective strategies to enhance soil health and sustainability in agricultural systems, ultimately contributing to climate change mitigation and more resilient and productive landscapes.

## 2. METHODS

### *2.1 Study fields*

The study was conducted on 22 farms located within the Cheney Watershed in central Kansas, with all farms situated within a 60-km radius (Fig. 4.1). Soils in the study region are fine-loamy, mixed, superactive, mesic Pachic Argiustolls primarily composed of Funmar-Tarver loam, Shellabarger sandy loam, and Hayes-Solvay loamy fine sand (1-5 % slope). The climate is characterized by a mean annual temperature of 13.2 °C and a mean annual precipitation of 777.5 mm. The soil pH across these sites ranged from 4.63 to 6.74. The soil texture varied significantly, with sand content ranging from 8.4% to 83.8%, and bulk density ranging from 1.12 to 1.9 g $cm^3$. These farms exhibited a range of management practices, including conventional 'control' farms and those implementing a variety of regenerative practices. The primary cash crop across all farms was rainfed wheat, but other crops including soybean, corn, pea, milo, sorghum, alfalfa, canola and rye were grown on some of the fields at some point in the last 10 years. Out of the 24 farms that opted in to the GMI initiative, we selected 22 farms , each with one field designated as a study field where both soil carbon and soil fauna sampling was conducted.

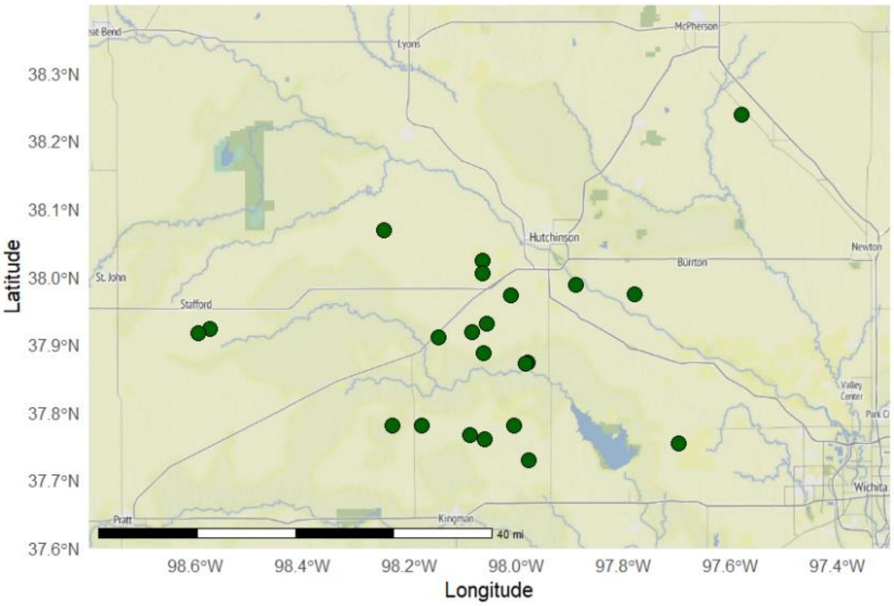

**Figure 4.1.** Map of the 22 producer fields sampled in this study.

### *2.2 Regenerative Farming Index*

Historical management data from 2010-2020 was collected for each farm to develop the Regenerative Farming Index (RFI). The data included information on crop diversity, cover crop usage, tillage disturbance, cropping system intensity, and livestock integration. We collected data on pesticide, herbicide, and fertilizer use but the data were too incomplete to include in the index.

The RFI was calculated based on composite scores derived from multiple management practices, allowing for a comprehensive, standardized, and easily interpretable measure that integrates diverse aspects of regenerative farming. Composite scores have been used in many fields to integrate complex and multidimensional data. For instance, in social sciences, sustainability science, and agricultural studies, composite indices help summarize social phenomena (Nardo et al., 2008), aggregate environmental, economic, and social dimensions into a single index (Singh et al., 2009), and evaluate farming practices by considering factors such as soil health, crop yield, and environmental impact (Tilman et al., 2011; Talukder et al., 2017; T. L. D. Fenster et al., 2021).

In this study, each practice was scored and normalized to a scale of 0 to 2, ensuring that the overall index was comprehensive and comparable across different farms. The following steps were undertaken to calculate the composite scores:

1. Crop diversity score

Crop diversity was calculated based on the number of crop species, including cover crops, grown in rotation. The diversity score was min-max normalized on a scale of 0 to 2 using the following formula:

$$Diversity\ score = \frac{diversity - 1}{max\ diversity - 1} \times 2$$

2. Cover crop score

Cover crop usage was scored categorically, with 2 points assigned if cover crops were used every year and 0 points if they were not. There was not enough information on cover crop variety and establishment to develop a more nuanced score.

3. Tillage intensity rating (gTIR) score

The general-purpose Tillage Intensity Rating (gTIR) was derived from the USDA-NRCS's STIR and modified by the Soil Health Institute (SHI) to measure the degree of soil disturbance caused by tillage equipment (*unpublished*). It accounts for all implements used and includes no-till seeder but excludes livestock grazing disturbance. The average gTIR per year is calculated as follows:

$$gTIR = 3.25 \times T \times D \times A$$

Where $T$ is the tillage type modifier, $D$ is the tillage depth, and A is the area of soil disturbed per pass (percentage). Tillage type modifiers depend on the classification of the implement including inversion, mixing, lifting/fracturing, compression. All implements used in a year are added together. A farm was only considered no-till if implemented for at least 10 years.

The gTIR score was min-max normalized, inversed, and scaled to 2, with higher scores indicating less disturbance:

$$gTIR\ score = \left(1 - \frac{gTIR}{max\ gTIR}\right) \times 2$$

The gTIR was compared against a categorical tillage score variable (NT, MT, CT) but performed better in variable importance tests.

4. Cropping intensity score

Crop intensity was based on the number of cash crops grown per year and was scored as 1 for single cropping and 2 for double cropping (e.g., wheat and soybean double crop).

5. Livestock integration score

Livestock integration was assessed through several components based on core metrics of grazing patterns (Stanley et al., 2024). The basic presence of livestock was scored as 1 point if present and 0 points if not. The intensity of livestock management was scored as 1 point for high intensity (high-density, rotational grazing) and 0.5 points for low intensity (low-density, continuous grazing). The timing of grazing was scored as follows: 0.25 points for fall grazing, 0.5 points for spring and fall grazing, 0.75 points for spring and summer or summer and fall grazing, and 1 point for spring, summer, and fall grazing. These values were chosen based on the type of cover (i.e. crop residue or cover crops) and length of time grazing occurred. The overall livestock score was the sum of each score normalized to a total possible score of 2:

$$livestock\ score = \left(\frac{livestock\ basic\ score + livestock\ intensity\ score + livestock\ timing\ score}{3}\right) \times 2$$

6. Regenerative Farming Index

Finally, the composite Regenerative Farming Index (RFI) was calculated by summing the individual scores for each management practice, resulting in a possible maximum score of 10.

$$RFI = diversity\ score + cover\ crop\ score + gTIR\ score + intensity\ score + livestock\ score$$

### *2.3 Soil fauna sampling and identification*

Soil fauna communities were sampled in each field in June and July along 150 m transects. Soil cores (10 cm diameter, 10 cm deep) were extracted three in three locations (50 m, 100 m, and 150 m marks) along the two transect lines in each field (n = 6 soil cores/field) and transported to Blue Dasher Farm for soil macrofauna extraction. Upon return to the laboratory, cores were placed in a Berlese funnel extraction system for 7 days, which permitted each soil core to completely dry and all arthropods to evacuate from the core (Pecenka and Lundgren, 2018). Upon completion of the Berlese system arthropods were stored in 70% isopropyl alcohol, until they could be identified and cataloged. Since sampling effort was equal across fields, we report total abundances summed from all 6 cores per field.

Each arthropod specimen collected was identified to the lowest taxonomic level feasible. Due to limitations in taxonomic references and time constraints, certain groups such as mites (Arachnida: Acari), Protura, thrips (Insecta: Thysanoptera), Symphyla, millipedes (Diplopoda: Julida), Diplura, and springtails (Hexapoda: Collembola) were identified only to their respective class or order. Other specimens were identified to the genus or species level and assigned a morphospecies identification number. Larvae of holometabolous insects were considered separate morphospecies from adults due to their different ecological roles. Based on current knowledge and ecological hypotheses, each morphospecies was categorized into one of seven functional guilds. The seven guilds were: detritivore, fungivore, omnivore, predator, herbivore, mite, and ant. Since we were unable to identify mite to family, they were considered a unique group due to their remarkable diversity and abundance. Ants were also categorized separately because of their unique and specific roles, including ecosystem engineering and eusociality. Voucher specimens are housed in the Mark F. Longfellow Collection at Blue Dasher Farm (Estelline, South Dakota, USA). All sampling, processing, and identification of arthropods was done by Ecdysis Foundation.

Family-level diversity was assessed using three primary metrics: family richness, Shannon-Weiner diversity index (Shannon's H’ index; Shannon, 1948), and Hill numbers (Hill 1 and Hill 2; Hill, 1973). Family richness, the simplest measure of diversity, represents the total number of different families present in the sample, providing a straightforward count of distinct family taxa without considering their relative abundances.

Shannon's H’ index accounts for both the abundance and evenness of families in a sample. It is calculated using the formula:

$$H' = -\sum_{i=1}^{S} p_i \ln(p_i)$$

where $S$ is the total number of families (family richness) and $pi$ is the proportion of individuals belonging to the $i$-th family. The Shannon index increases with both the number of families and the evenness of their distribution.

Hill numbers, also known as the effective number of species, provide a unified approach to measuring diversity that includes family richness and Shannon's H' index as special cases (Hill, 1973). Hill numbers of order $q$ are defined as:

$$^{q}D = \left(\sum_{i=1}^{S} p_i^{q}\right)^{\frac{1}{1-q}}$$

where $q$ determines the sensitivity to rare versus common families. For this study, we used Hill 1 and Hill 2 diversity. Hill 1 diversity, equivalent to the exponential of Shannon's entropy, provides a measure of diversity (D) more sensitive to the evenness of the distribution, or the effective number of rare species, and is calculated as: $^{1}D = e^{H'}$ where $H$' is Shannon's H' index. Hill 2 diversity, equivalent to the inverse of Simpson's index, is less sensitive to rare families and focuses more on the dominant families in the community. It is calculated using the formula:

$$^{2}D = \left(\sum_{i=1}^{S} p_i^{2}\right)^{-1}$$

The use of Hill numbers allowed for a nuanced interpretation of diversity by balancing sensitivity to rare and common families.

### *2.4 Soil Sampling*

Soil sampling was conducted by Resource Environmental Solutions (RES) in March 2020 using a Giddings hydraulic sampling unit mounted on a Polaris ATV. Sampling points were selected using a conditioned Latin Hypercube Stratification (cLHS) algorithm (Minasny and McBratney, 2006), which allocated 27 soil cores per field based on multiple data layers including texture, NDVI, slope, and soil type to characterize field variability (we selected 594 of 2592 total cores). Soil cores were delivered to the University of Missouri's Soil Health Assessment Center (SHAC) for core descriptions, splitting, and compositing. Cores were divided into four depth increments: 0-15 cm, 15-30 cm, 30-50 cm, and 50-100 cm. For this study, only the 0-15 cm and 15-30 cm increments were used. SHAC conducted measurements of bulk density, total carbon by dry combustion, and inorganic carbon by pressure calcimeter, with the organic carbon obtained by difference (REF). One sample per stratum was also analyzed for texture (particle size determination) and pH. The 0-15 cm and 15-30 cm increments were pressed through an 8-mm sieve. After air drying, a representative 50 g subsample from the 8-mm-sieved samples was placed into a 2-mm sieve and shaken, with any remaining soil gently ground with a mortar and pestle to pass through the sieve. The 2-mm air-dried samples were shipped to Colorado State University for further size fractionation for POM and MAOM.

### *2.5 POM and MAOM fractionation*

The size fractionation method employed in this study was adapted from Cotrufo et al. (2019) as described in Leuthold et al. (2022). An 8 g subsample of the 2 mm air-dried soil was oven-dried overnight at 60°C to remove residual moisture. To disperse soil aggregates, 35 mL of 0.5% sodium hexametaphosphate (SHMP) solution and twelve 4 mm glass beads were added to the subsample. The samples were shaken at 112 rpm for 18 hours on a reciprocal shaker. The dispersed soil was then rinsed onto a 53 µm sieve. Material that passed through the sieve was collected as MAOM (<53 µm), and material retained on the sieve was collected as POM (>53 µm). All 27 soil samples per field from the 0-15 cm depth increment were size fractionated as described. Due to resource limitation, 10 samples per field were fractionated for the 15-30 cm depth increment. This subset was selected to represent an even distribution of total SOC values, to ensure that the samples captured the field variability in SOC content. Recoveries of the initial soil masses in the summed fractions were +/- 5% for all samples. Soils containing carbonates (identified by effervescence after addition of 5% HCl, 32 of 814 samples) were treated to remove inorganic carbon via HCl fumigation (Harris et al., 2001). Percent organic C and total N were analyzed by vario ISOTOPE CUBE elemental analyzer (EA; Elementar Langenselbold, Germany). The average recovery of initial C and N in fractions was 99.2 ± 6.0 % C and 92.6 ± 2.9 % N. Concentrations of POM and MAOM were calculated by multiplying the relative percent C or N of the fraction by the proportion of mass made up by that fraction. Carbon stocks (Mg C $ha^{-1}$) of SOM, MAOM, and POM were calculated as follows:

$$C_s = (C_c * BD * depth)/10$$

where $C_s$ is the C stock in Mg C $ha^{-1}$, $C_c$ is the C concentration in g C kg $soil^{-1}$, *BD* is the bulk density in g $cm^{-3}$, and *depth* is the soil sampling depth increment in cm.

### 2.6 Estimation of C saturation and saturation deficit of MAOM

To estimate the theoretical C saturation in MAOM, we adapted the approach from King et al. (2024) and used the dataset from Georgiou et al. (2022) along with our own data. We utilized the dataset of Georgiou et al. (2022) to establish a theoretical maximum due to its comprehensive representation of native vegetation, which typically exhibits higher C levels in MAOM than agricultural soils. Given that our Mollisol soils are characterized by high activity minerals, we filtered the dataset to include only soils with this mineralogy (*sensu* Georgiou et al., 2022). For the boundary line analysis, we performed a quantile regression for the 95th percentile forced to zero to estimate the boundary line slope of MAOM (g C kg $soil^{-1}$) as a function of silt and clay content using the ‘quantreg’ package (Koenker et al., 2024). This analysis resulted in a boundary line slope of 0.7432. Using this slope, we calculated the theoretical maximum C in MAOM for our samples by multiplying the silt and clay content by 0.7432. To assess the *C saturation deficit of the MAOM*, we calculated the absolute deficit by subtracting the observed C from the theoretical C, and the *percent C saturation* by dividing the observed C by the theoretical C and multiplying by 100.

### *2.7 Statistical analyses*

All statistical analyses, data manipulation, and data visualization were performed on R software version 4.4.0 (R Core Team, 2020) with the R packages *dplyr* (Wickham et al., 2019) and *ggplot2* (Wickham et al., 2019). To assess the relative importance of different management practices and soil properties in predicting SOC content, we utilized a random forest regression approach using the *randomForest* package (Cutler and Wiener, 2022). The analysis included the following variables: RFI, diversity score, cover crop score, tillage intensity score (gTIR score), crop intensity score, livestock score, soil pH , and percent sand, with SOC stocks (MgC ha$^{-1}$) as the response variable. Variable importance was evaluated based on the mean increase in node purity, with higher values indicating greater predictive importance.

To assess the effect of management on soil fauna diversity, we performed linear regressions of total arthropod abundance, family richness, and family diversity (Shannon H', Hill 1, and Hill 2) with the RFI as a continuous predictor variable. Soil pH, percent sand, and percent soil moisture were included as fixed effects. Additionally, we categorized the RFI into two groups: 'High' (greater than 5) and 'Low' (less than 5). Nonmetric multidimensional scaling (NMDS) ordination was employed to evaluate differences in the variance and composition of fauna functional communities between the two management categories (High and Low). This analysis was conducted using the ‘betadisper’ and ‘adonis2’ functions in the *vegan* package of R (Oksanen et al., 2024). The model included soil pH, percent sand, percent SOC when assessing the composition of the different arthropod communities.

To assess the effect of management on C and N stocks (Mg $ha^{-1}$) of SOM, POM, and MAOM, we employed linear mixed-effects models using the *lme4* package (Bates et al., 2015,). The RFI served as the primary continuous predictor variable, with percent sand included as an additional fixed effect, and field code as a random effect. Similarly, we explored the relationship between management and *C saturation deficit* and *percent C saturation of the MAOM fraction* using linear mixed-effects models with field code as a random effect. Variables were log-transformed as necessary to meet the assumptions of homogeneity of variance and normality of residuals. Diagnostic plots of all models were produced to check assumptions of normality, homoscedasticity, and linearity. Full and reduced mixed effects models were compared with Wald Chi-Square tests to approximate p-values. For all statistical analysis, $p < 0.05$ was considered significant and $p < 0.1$ as marginally significant for biodiversity metrics.

Using results from our linear regressions, we conducted a path analysis using the *lavaan* package (Rosseel, 2012) to investigate direct and indirect effects of the RFI, soil pH, and soil texture (percent sand) on soil fauna diversity (Hill 1 diversity), *C saturation deficit,* and POM N. The model fit was evaluated using Chi-square test ($p > 0.05$), Comparative Fit Index (CFI > 0.90), Root Mean Square Error of Approximation (RMSEA < 0.08), and Standardized Root Mean Square Residual (SRMR < 0.08) (Hooper et al., 2008). Path coefficients and indirect effects were calculated to determine the relationships among variables (Grace et al., 2010; Shipley, 2016).

## 3. RESULTS

### *3.1 Regenerative Farming Index*

The Regenerative Farming Index (RFI) of the 22 farms in our study ranged from 1 to 9.4, with a median score of 5.8 (Fig. 4.2).

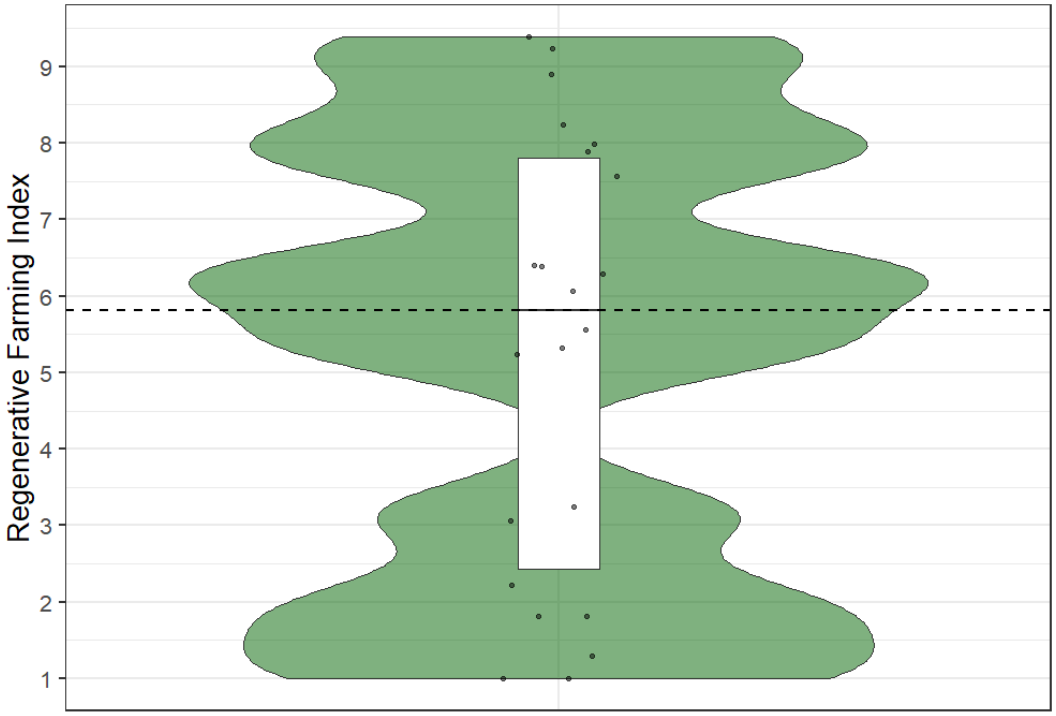

**Figure 4.2.** The distribution of Regenerative Farming Index scores across the study fields using a violin plot with an embedded box plot. The violin plot shows the density of scores, with wider sections indicating a higher concentration of values. The box plot within the violin plot displays the median and upper and lower interquartile ranges. The dashed horizontal line indicates the median score across all farms.

### *3.2 Influence of Regenerative Farming Index on soil fauna abundance, diversity, and functional composition*

Total soil fauna abundance (sum of 6 cores) across farms ranged from 5 to 873 individuals, with a mean of 187.3. there was no significant relationship between total soil fauna abundance and RFI (t = -0.90, *p* = 0.381). Family-level richness, however, was positively correlated with RFI (t = 2.98, *p* = 0.008), with an average increase in richness of 1 family for every 1.25-unit increase in RFI. In addition, the RFI was strongly correlated with soil fauna family diversity, as measured by Shannon's H' (t = 3.91, p = 0.001) and Hill 1 (Fig. 4.3A; t = 5.22, p < 0.0001). However, no significant correlation was found with Hill 2

(Fig. 4.3B; t = -0.792, p = 0.439), suggesting that the RFI has a distinct effect on rare species rather than abundant species.

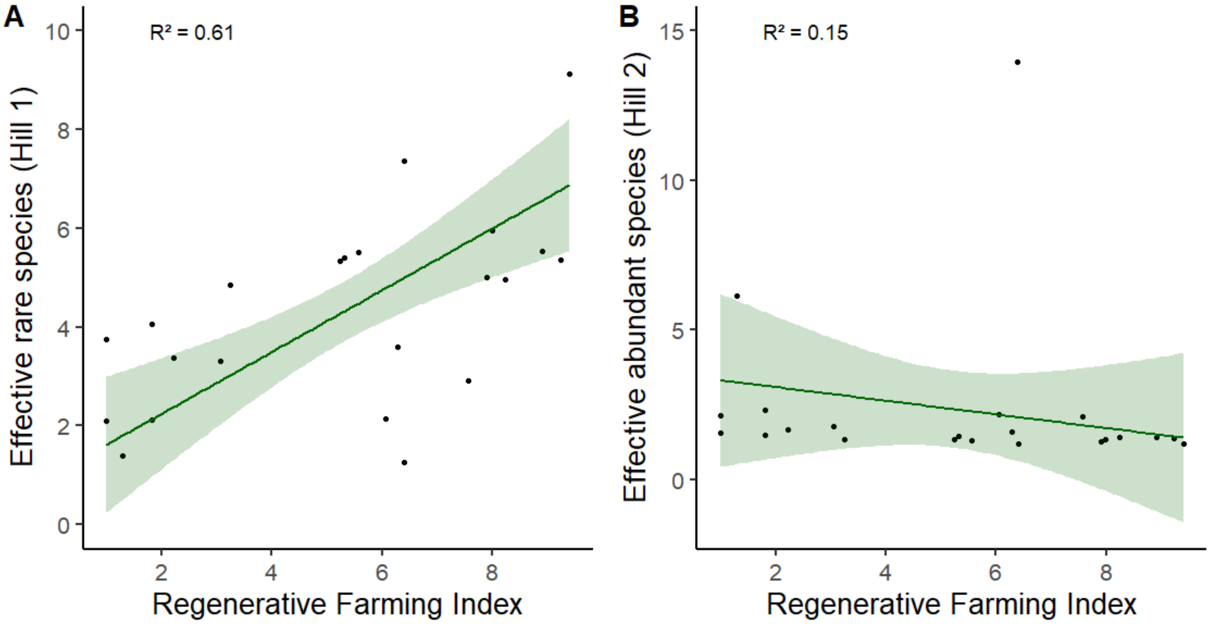

**Figure 4.3.** The relationship between the Regenerative Farming Index (RFI) and soil fauna diversity metrics. (**A**) Effective rare species as measured by Hill number 1 and (**B**) Effective abundant species as measured by Hill number 2 are plotted against the RFI. The green line represents the linear fit from the model, with the shaded area indicating the 95% confidence interval. Higher values on the y-axis indicate greater diversity. The RFI was a significant predictor of effective rare species (*p* = 0.0003) but not of effective abundant species.

To investigate the relative importance of various management and soil variables in predicting soil fauna diversity in topsoil (0-10 cm), we employed a random forest algorithm (Fig. 4.4). Our analysis revealed that the RFI explained the most variation in soil fauna familial diversity (Shannon H') compared to other individual management scores, soil pH, and percent sand (Fig. 4.4). Aside from RFI, individual scores related to livestock integration and tillage management had the most significant influence on soil fauna diversity.

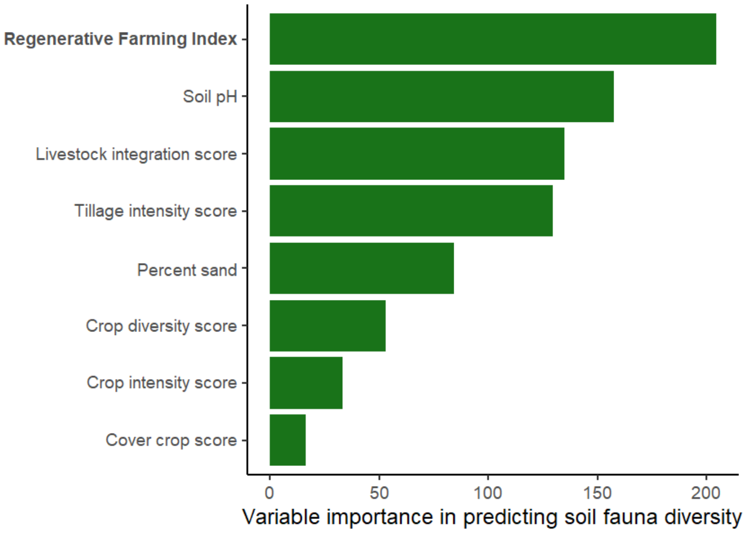

**Figure 4.4.** The importance of various predictor variables in a random forest model for predicting topsoil (0-10cm) soil fauna familial diversity (Hill 1). The x-axis represents the mean increase in node purity, indicating the importance of each variable. Higher values suggest greater importance.

When we split the Regenerative Management Index into two groups, 'High' (RFI > 5) and 'Low' (RFI < 5), we identified two distinct populations of organisms. The NMDS analysis of soil fauna community converged on solutions with stress values of 0.22 and 0.18 for family composition and functional composition, respectively. The NMDS analysis of family composition showed marginally significant heterogeneity of dispersion (F = 1.68, p = 0.093), suggesting a slightly distinct beta diversity of community composition between High and Low RFI groups. In contrast, the NMDS analysis of functional composition revealed significant heterogeneity of dispersion (F = 2.28, p = 0.049), indicating distinct functional compositions between High and Low RFI groups (Fig. 4.5).

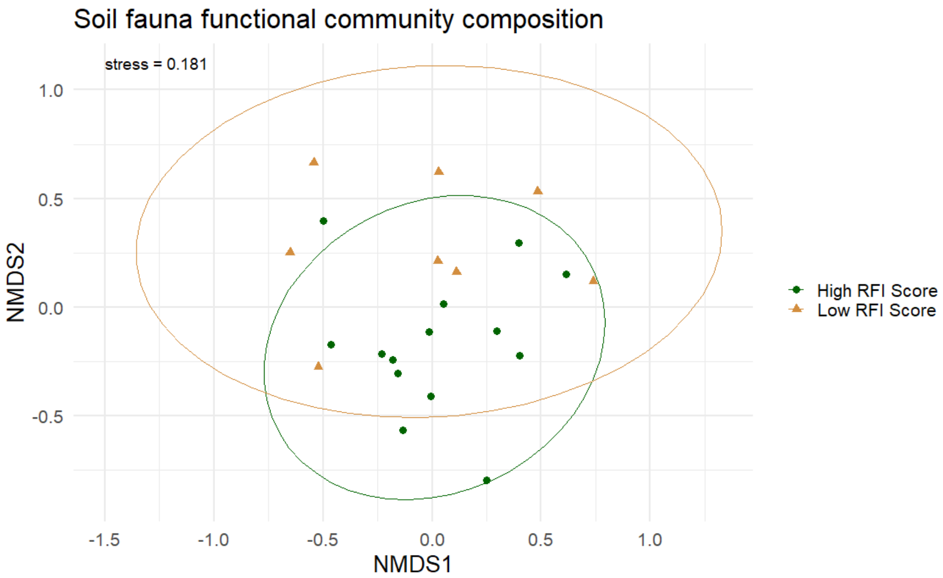

**Figure 4.5.** Nonmetric multidimensional scaling (NMDS) plot for soil functional-level community composition. The ellipses represent 95% confidence intervals for Regenerative Farming Index (RFI) score groups: 'High' (RFI > 5) and 'Low' (RFI < 5). The seven functional guilds of soil fauna included detritivore, fungivore, omnivore, predator, herbivore, mite, and ant. The stress value for the NMDS analysis is indicated in the upper left corner of the plot, reflecting the goodness-of-fit of the ordination. A stress value below 0.2 is considered an "acceptable" fit.

Average soil fauna abundance in High RFI fields was 199.5 ± 66.7 individuals (mean ± se) while the average abundance in Low RFI fields was 166.0 ± 42.0. Furthermore, the average abundances of the seven functional guilds varied significantly between High and Low RFI groups (Fig. 4.6). In particular, the ant ($p = 0.026$), herbivore ($p = 0.102$), mite ($p = 0.094$), omnivore ($p = 0.073$), and predator ($p = 0.106$) groups showed significant differences in their abundances between the two RFI groups. There were 24 unique families in farms with a High RFI scores, including 9 predators, 2 omnivores, 6 herbivores (pests), 6 detritivores, and 1 fungivore. In contrast, there were only 3 families unique to farms with a Low RFI score, comprising 1 pest and 2 predators.

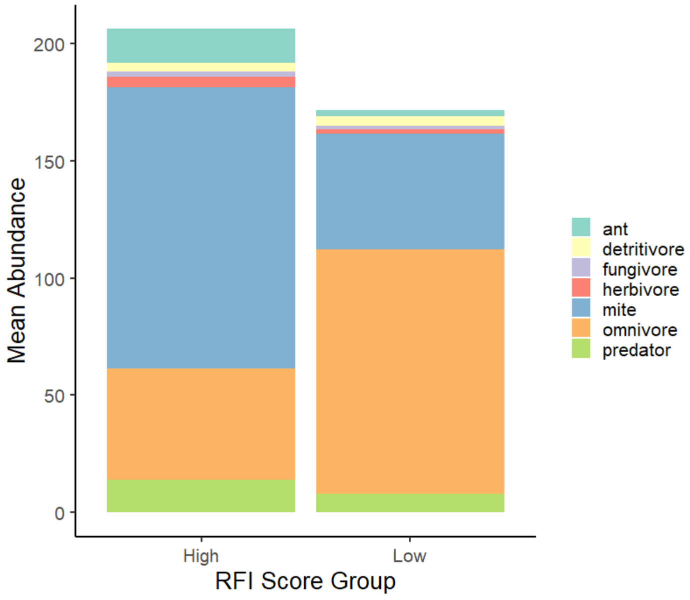

**Figure 4.6.** Mean abundance of soil fauna functional guilds for each Regenerative Farming Index (RFI) score group. The groups are categorized as 'High' (RFI > 5, n = 14) and 'Low' (RFI < 5, n = 8).

### *3.3 Regenerative Farming Index as a predictor of SOM, POM, MAOM, and C saturation*

In the 0-30 cm soil profile, there was no significant relationship between the RFI and bulk soil organic carbon (SOC) stocks ($t = 1.65$, $p = 0.115$), mineral associated organic carbon (MAOC) stocks ($t = 1.71$, $p = 0.104$), or particulate organic carbon (POC) stocks ($t = 1.14$, $p = 0.269$) (data not shown). However, the topsoil (0-15 cm), SOC, MAOC, and POC stocks (Mg C ha$^{-1}$) all showed significant positive relationships with RFI (Fig. 4.7A). Specifically, bulk SOC increased by 0.75 ± 0.34 Mg C ha$^{-1}$ (mean ± se; $t = 2.29$, $p = 0.034$), MAOC by 0.55 ± 0.29 Mg C ha$^{-1}$ ($t = 2.19$, $p = 0.041$), and POC by 0.21 ± 0.07 Mg C ha$^{-1}$ ($t = 3.03$, $p = 0.007$) for every one-unit increase in RFI. This equated to a 3.9% (95% CI: 0.6 – 7.3%), 3.8% (95% CI: 0.4 – 7.4%), and 6.2% (95% CI: 2.1 – 10.4%) increase in SOC, MAOC, and POC stocks for every one-unit increase in RFI.

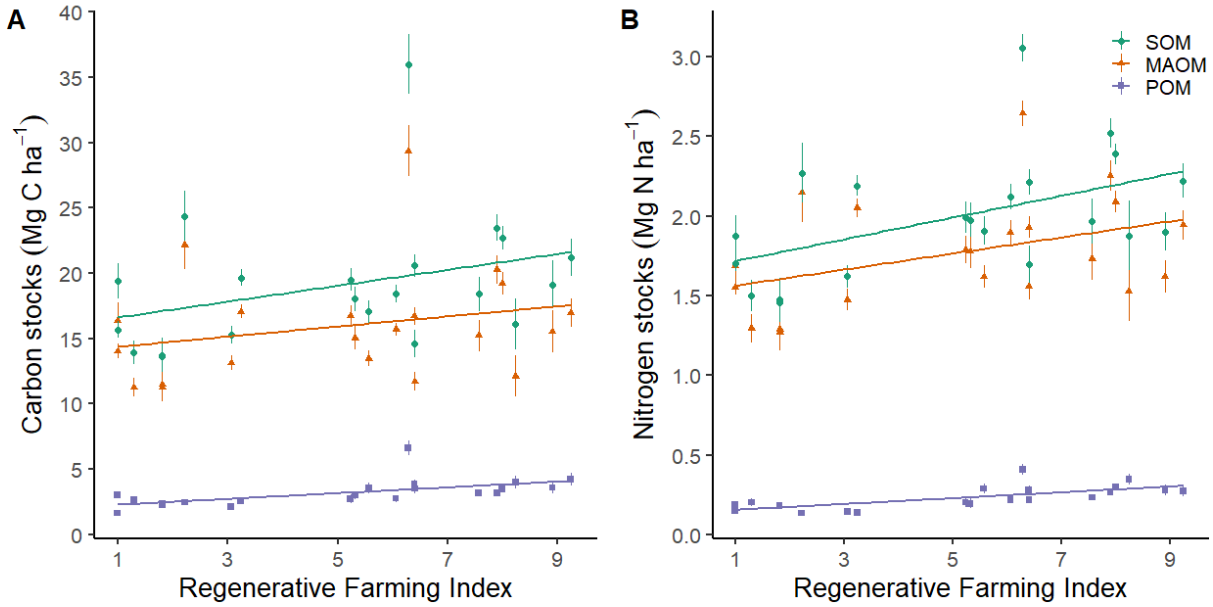

**Figure 4.7.** The relationship between the Regenerative Farming Index (RFI) and (**A**) organic carbon stocks (Mg C ha$^{-1}$) and (**B**) nitrogen stocks (Mg N ha$^{-1}$) in topsoil (0-15 cm). The points represent the mean values for each farm, and the error bars indicate the standard error. Lines represent the linear regressions for soil organic matter (SOM), mineral-associated organic matter (MAOM), and particulate organic matter (POM). The RFI was a significant predictor of both carbon and nitrogen stocks in SOM, MAOM, and POM ($p < 0.05$).

To investigate the relative importance of various management and soil variables in predicting SOC, MAOC, and POC stocks (Mg C ha$^{-1}$) in topsoil (0-15cm), we employed a random forest algorithm (Fig. 4.9). For bulk SOC stocks, the random forest analysis indicated that while the RFI explained more variation in SOC stocks than any single management practice, it was less influential than soil pH and percent sand (Fig. 4.3A). For MAOC stocks, soil pH and percent sand also explained the most variation, followed by RFI, livestock integration score, and crop diversity score (Fig. 4.8B). Soil pH was the biggest predictor of POC stocks, with tillage intensity having the largest management impact, followed closely by RFI (Fig. 4.8C).

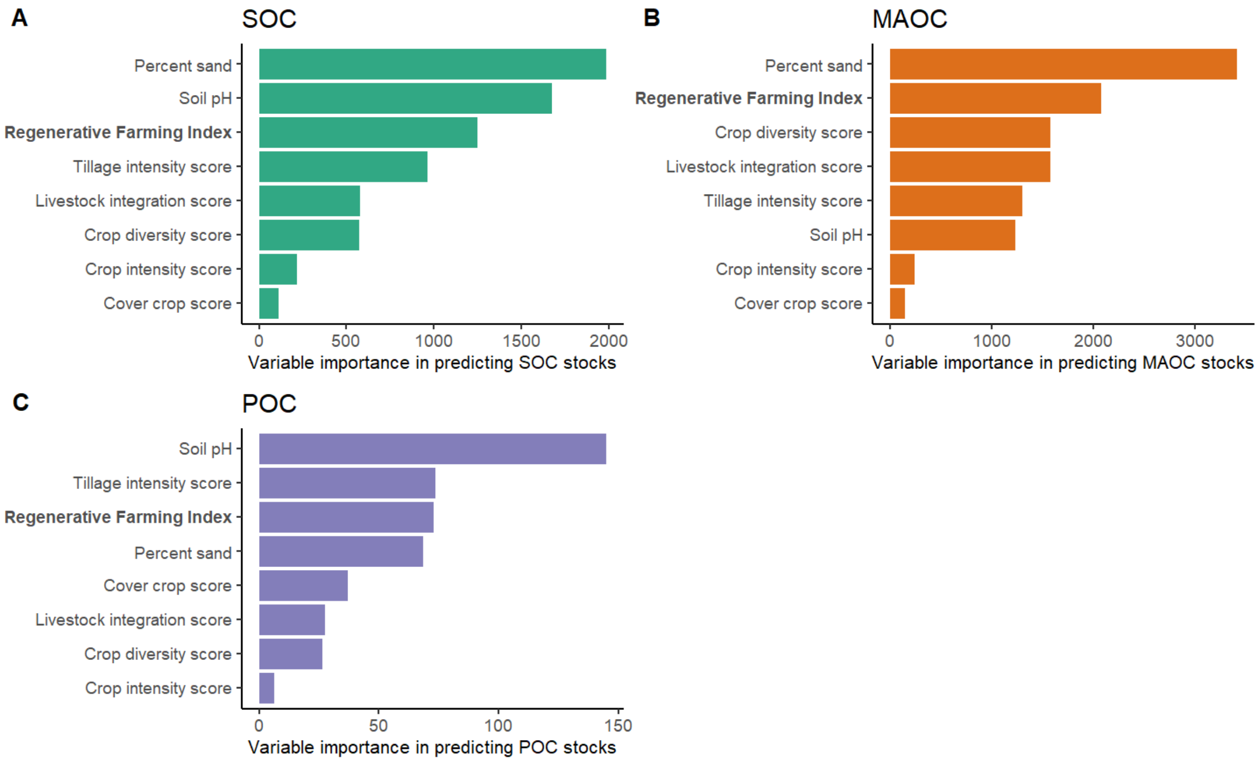

**Figure 4.8.** The importance of various predictor variables in a random forest model for predicting topsoil (0-10cm) (**A**) soil organic carbon (SOC) stocks (Mg C ha$^{-1}$), (**B**) mineral-associated organic matter organic carbon (MAOC) stocks (Mg C ha$^{-1}$), and (**C**) particulate organic carbon (POC) stocks (Mg C ha$^{-1}$). The x-axis represents the mean increase in node purity, indicating the importance of each variable. Higher values suggest greater importance.

Furthermore, the stocks of N increased by 0.08 ± 0.02 Mg N ha$^{-1}$ in SOM ($t$ = 3.13, $p$ = 0.005), by 0.06 ± 0.02 Mg N ha$^{-1}$ in MAOM ($t$ = 2.78, $p$ = 0.012), and 0.02 ± 0.005 Mg N ha$^{-1}$ in POM ($t$ = 3.54, $p$ = 0.002) for every one-unit increase in RFI (Fig. 4.7B). This equated to a 4.2% (95% CI: 1.5 – 7.0%), 5.8% (95% CI: 1.9 – 9.9%) , and 6.9% (95% CI: 3.2 – 10.8%) increase in SOM, MAOM, and POM N stocks for every one-unit increase in RFI.

The relationship between RFI and MAOC in topsoil was further highlighted when considering *C saturation deficit* (Fig. 4.9A) and *percent C saturation* (Fig. 4.9B) in MAOM. A one-unit increase in RFI scores was associated with a 2.09 ± 0.72 g C kg soil$^{-1}$ decrease in *C saturation deficit* (Fig. 4.9A; $t$ = -

2.89, $p = 0.009$) and a 1.57 % (95% CI: 0.41 – 2.52%) increase in *percent C saturation* (Fig. 4.9B ; $t = 2.72$, $p = 0.014$).

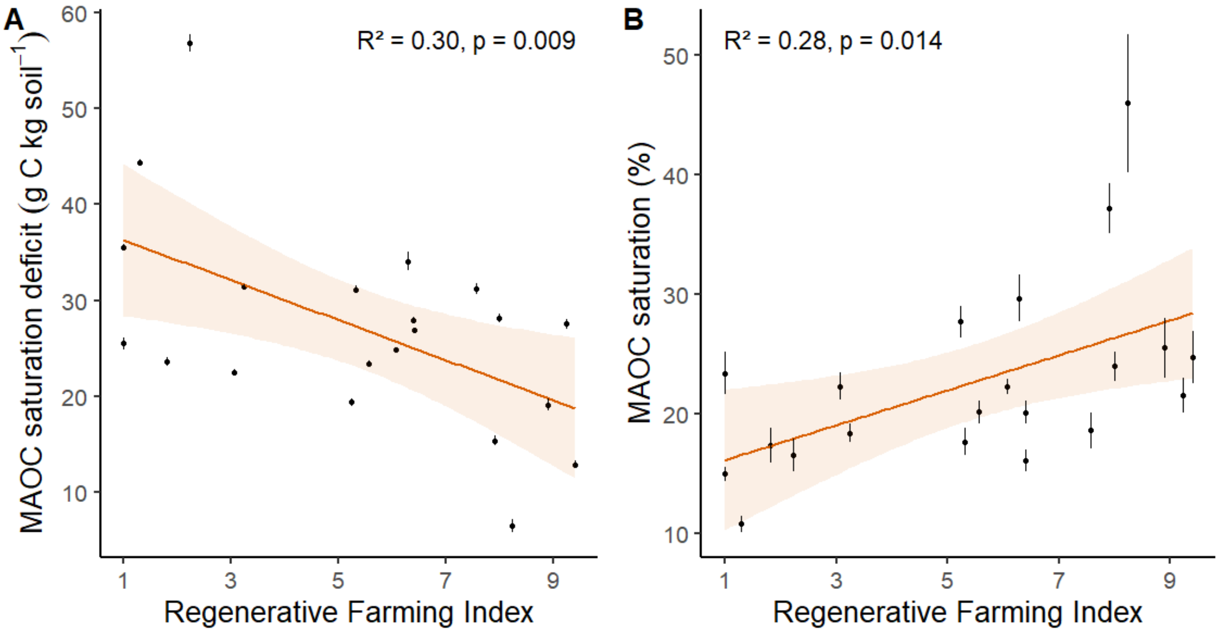

**Figure 4.9.** The relationship between the Regenerative Farming Index (RFI) and (**A**) *C saturation deficit* (g C kg soil$^{-1}$) and (**B**) *percent C saturation* (%) in topsoil (0-15 cm) mineral associated organic matter fraction. The points represent the mean values for each farm, and the error bars indicate the standard error. Lines represent the linear regressions with the $R^2$ and p-value associated displayed in the upper corners of each plot. The RFI was a significant predictor of *C saturation deficit* and *percent C saturation* ($p < 0.05$).

### *3.4 Biodiversity and SOM relationships*

Significant relationships between soil biodiversity metrics and SOM were observed only with C saturation deficit. Specifically, C saturation deficit was significantly correlated with Hill 1 (Fig. 4.10; estimate = -2.49, se = 1.14, $t = -2.20$, $p = 0.041$) and Shannon's H' (estimate = -8.74, se = 4.37, $t = -2.00$, $p = 0.06$). No other significant correlations were found between SOM, MAOM, or POM C or N stocks, concentrations, or C:N ratio.

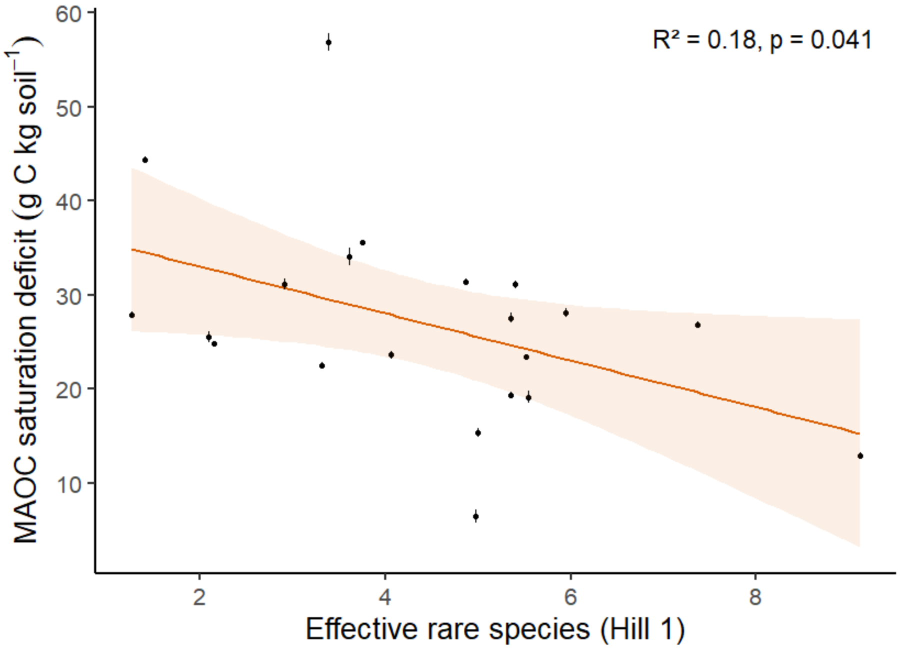

**Figure 4.10.** The relationship between effective rare species as measured by Hill number 1 and *C saturation deficit* (g C kg soil$^{-1}$) in topsoil (0-15 cm) mineral associated organic matter fraction. The points represent the mean values for each farm, and the error bars indicate the standard error. Lines represent the linear regressions with the $R^2$ and p-value associated displayed in the upper corners of each plot. Hill 1 was a significant predictor of *C saturation deficit* ($p < 0.05$).

### *3.5 Path analysis*

To assess the linkages between management, soil fauna diversity, and POM and MAOM, we constructed a path analysis testing RFI against the most responsive variables: Hill 1 diversity, POM N stocks (Mg N ha$^{-1}$), and MAOM C saturation deficit (g C kg soil$^{-1}$) (Fig. 4.11). Environmental variables including soil pH and percent sand were also included. Our path analysis fit the data well ($\chi^2 = 0.284$, $p = 0.594$; CFI = 1.0; RMSEA = 0.0002; SRMR = .021; Hooper et al., 2008) and explained 44.6% of the variation in soil fauna diversity (Hill 1), 68.2% of the variation in POM N, and 43.5% of the variation in MAOM C saturation deficit. We structured the path analysis to have Hill 1 diversity predict POM N stocks and MAOM C saturation deficit, rather than the reverse, as this model configuration yielded a better fit. The direction of causation remains unclear. In line with our linear regression results, RFI positively influenced Hill 1 diversity and POM N stocks, and negatively influenced MAOM C saturation deficit. Additionally, soil pH had a positive relationship with POM N and a negative relationship with Hill 1 diversity, and percent sand strongly predicted MAOM C saturation deficit. Contrary to our linear regression findings, Hill 1 did not significantly predict MAOM C saturation deficit in the path analysis. This discrepancy is likely due to the effect of RFI on both variables, as path analysis calculates partial regression coefficients that account for correlations and covariance between multiple variables.

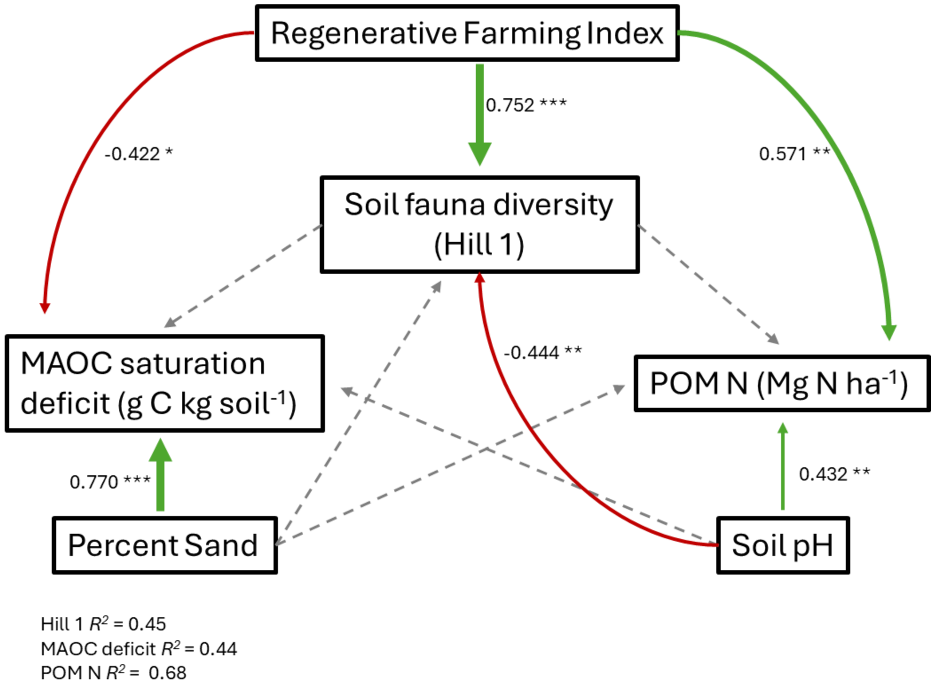

**Figure 4.11.** Results from path analysis showing linkages between Regenerative Farming Index (RFI), soil fauna diversity (Hill 1 diversity), soil pH, percent sand, particulate organic matter (POM) nitrogen stocks (POM N; Mg N ha$^{-1}$), and mineral associated organic matter (MAOM) C saturation deficit (g C kg soil$^{-1}$). Green and red arrows indicate significant positive and negative effects, respectively, of the variables on Hill 1 diversity, MAOM C saturation deficit, and POM N stocks. Grey, dashed arrows indicate non-significant paths. The widths of the arrows correspond to standardized path coefficients, shown in numbers beside each arrow. Significance of each pathway is denoted by ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. The $R^2$ values in the bottom left corner indicate the amount of variation explained by direct and indirect effects of path analysis on Hill 1, MAOM C saturation deficit, and POM N stocks.

# 4. DISCUSSION

Our study underscores the importance of regenerative agriculture in supporting soil biodiversity and SOM dynamics. Real-world conditions are unpredictable, and successful agricultural management must be adaptive to these conditions. By studying real-world farms, we can gain a more holistic understanding of how management influences soil health. Management scenarios rarely fall into neat, discrete categories such as conventional and regenerative. Our objective in creating a continuous index that captures the gradient of management practices was to account for this wide diversity in strategies (Fig. 4.2), even within the same region (Fig. 4.1). Our findings indicate that adopting multiple regenerative agricultural practices in combination can lead to notable improvements in soil fauna diversity, POM, and MAOM within the top 15 cm of soil. These results align with previous studies suggesting that stacking multiple soil health practices yields greater benefits for soil biodiversity and SOM dynamics (Lehman et al., 2019; Prairie et al., 2023; Villat and Nicholas, 2024).

## *4.1 Regenerative management increases rare soil fauna and shifts functional composition*

Our analysis revealed a strong positive relationship between the RFI and soil fauna diversity, specifically family richness and diversity metrics such as Shannon's H' and Hill 1 (Fig. 4.3). These metrics showed a significant increase with higher RFI scores, indicating that regenerative practices foster a more diverse soil fauna community, favoring rare species over abundant ones. This is consistent with studies that have highlighted the role of diverse cropping systems, reduced tillage, cover crops, and integrated livestock in enhancing soil biodiversity (Lemaire et al., 2014; Rosenzweig et al., 2018; de Graaff et al., 2019; Bansal et al., 2022; Bier et al., 2024; Cozim-Melges et al., 2024).

The random forest indicated a larger effect of tillage management and livestock integration on soil fauna diversity than the other practices (Fig. 4.4). Tillage can significantly disrupt soil fauna through changes in soil structure and direct mortality. It breaks up soil aggregates, exposing soil fauna to predators and environmental stressors, while also physically killing individuals during the tillage process (van Capelle et al., 2012; Betancur-Corredor et al., 2022). These disruptions can reduce the habitat availability and stability required for diverse soil fauna communities, leading to declines in biodiversity (Rizk and Mikhail, 1999), with some organisms more resilient to disturbance than others (Coulibaly et al., 2022). Livestock integration introduces specialist coprophagic species due to the presence of manure, which can directly increase soil fauna diversity (Schmid et al., 2024). Manure also provides a rich organic substrate that can enhance soil fertility and supports the growth of various microorganisms and soil fauna (Rayne and Aula, 2020). Grazing can also influence root photosynthate allocation, providing a greater energy flux into the soil, potentially supporting more diverse soil communities (Acosta-Martínez et al., 2004; Piñeiro et al., 2009; de Faccio Carvalho et al., 2010; Tian et al., 2010; Brewer and Gaudin, 2020; Alves et al., 2023).

The NMDS analysis further demonstrated distinct functional compositions between high and low RFI groups (Fig. 4.5), suggesting that regenerative practices not only increase diversity but also shift the functional structure of soil communities. The significant differences in the abundance of functional guilds, such as ants, herbivores, mites, omnivores, and predators, highlight the potential of regenerative practices to enhance ecosystem functions, including pest control and nutrient cycling (Brussaard et al., 2007; Bardgett and van der Putten, 2014; Smith et al., 2015). Most notably, we observed a shift from omnivorous generalist families in low RFI fields to more specialist groups of predators and mesofauna, such as mites, in high RFI fields (Fig. 4.6). This shift suggests that regenerative practices create an environment that supports a higher diversity of specialized organisms. Although herbivore pest abundance and diversity were greater in more regenerative systems, the higher abundance and diversity of predators also indicated a greater potential for biocontrol. This finding highlights the potential for regenerative practices to contribute to a more resilient agroecosystem with greater redundancy of biodiversity-driven ecosystem functions (Liebman and Schulte, 2015; Martin et al., 2019; Dardonville et al., 2022).

### *4.2 Regenerative management enhances POM and MAOM*

In the top 15 cm of soil, we observed significant positive relationships between RFI and stocks of C in SOM, MAOM, and POM (Fig. 4.7). The increases in these C pools with higher RFI scores suggest that regenerative practices can enhance C accrual, contributing to climate change mitigation (Prairie et al., 2023; Rehberger et al., 2023). The relationship between RFI and MAOM was particularly evident when considering C saturation deficit and percent C saturation (Fig. 4.9). Higher RFI scores were associated with reduced MAOM C saturation deficit and increased percent C saturation, supporting the notion that soils farther from their C saturation have greater potential for carbon storage in MAOM (Georgiou et al., 2022; Angst et al., 2023). Though trends were positive, RFI did not significantly affect SOM down to 30 cm (data not shown). Since topsoil is more biologically active and susceptible to tillage disturbance, SOM takes longer to accumulate and is slower to be lost at depth, especially in agricultural systems (Knebl et al., 2017). It is possible that practice implementation has not been long enough to see an effect below 15 cm (Prairie et al., 2023) and certain practices (e.g., no-till) are unlikely to ever have impacts on C below 15 cm. Additionally, the lower number of samples for the deeper depth may have reduced the statistical power needed to detect small differences across farms.

Predictably, crop diversity and livestock integration pulled out as the two most influential management practices determining MAOC stocks (Fig. 4.8B). Diverse cropping systems, especially those including legumes, enhance input quality and promote MAOM formation efficiency due to higher nitrogen availability, which facilitates the decomposition and stabilization of organic inputs by soil microbes (Drinkwater and Snapp, 2007; Haddix et al., 2016; Giller et al., 2021; van der Pol et al., 2022). Additionally, diverse root systems can improve soil structure and increase root exudates, both of which contribute to the formation and stabilization of MAOM (Lange et al., 2015; Sokol et al., 2019a). Similarly, livestock integration continuously provides organic inputs through manure, promoting soil microbial communities that directly contribute to MAOM formation (Rayne and Aula, 2020). Grazing also increases root exudation (Piñeiro et al., 2009; Brewer and Gaudin, 2020), providing fresh, low molecular weight substrates that can improve microbial substrate use efficiency and MAOM formation (Tian et al., 2010; Cotrufo et al., 2013; Sokol et al., 2019b).

Furthermore, N stocks were more responsive to RFI than C stocks, particularly in POM, hinting at increased input quality and its implications for soil N cycling and soil health (Fig. 4.7) (Bu et al., 2015; Bailey et al., 2019; Cotrufo and Lavallee, 2022). POM plays a crucial role in providing C and nutrients for microbial energy use and plant growth, as it is more physically accessible for microbial utilization and serves as the primary energy source driving biological functions in the soil, including soil fauna activity (Merckx et al., 2001; Bailey et al., 2019; Lavallee et al., 2020). This makes POM a key indicator of soil health. Although the absolute increase in POM is less than that of MAOM due to its relatively small contribution to the total SOM pool, it experienced the greatest relative increase. Given that POM is an essential source of nutrients, our findings suggest that regenerative agriculture can enhance soil health and fertility by increasing POM C and N.

Given the sensitivity of POM to decomposition (Hansen et al., 2023), it is unsurprising that tillage intensity had the largest management effect on POC stocks (Fig. 4.8C). Tillage disrupts soil structure, leading to the breakdown of soil aggregates and exposing POM to decomposition (Six et al., 1999; Devine et al., 2014). Reduced tillage or no-till practices help preserve soil structure, maintain soil aggregates, and protect POM from rapid decomposition, thus enhancing POM stocks.

The potential synergies between reduced tillage, livestock integration, and crop diversity on both MAOM and POM are particularly noteworthy (Fig. 4.8A). Combining these practices can lead to enhanced POM protection and MAOM formation by increasing quality of inputs through livestock integration and crop diversity, while improving soil structure and protection from reduced tillage disturbance (Fig. 4.8A). This aligns with global patterns (Prairie et al., 2023), lending support to the finding that integrated livestock combined with no-till and systems diversified with cover crops significantly increase SOC, MAOC, and POC. This synergy underscores the importance of adopting multiple regenerative practices to achieve optimal soil health and carbon sequestration.

Additionally, soil pH and percent sand significantly influenced POM and MAOM, respectively, consistent with global patterns governing POM and MAOM accumulation (Hansen et al., 2024). Soil pH, which moderates decomposition, was found to influence POM, while percent sand directly predicted MAOM as MAOM must bind to silt and clay in the soil. The positive relationship between soil pH and POM suggests that pH affects the stability and turnover of POM by influencing microbial activity and decomposition rates (Rousk et al., 2009; Lugato et al., 2021; Cotrufo and Lavallee, 2022; Yu et al., 2022). Conversely, the strong influence of percent sand on MAOM highlights the importance of soil texture in determining the capacity for MAOM storage (Cotrufo et al., 2019; Georgiou et al., 2022; Mao et al., 2024).

### *4.3 Linkages between RFI, biodiversity, and SOM*

The path analysis provided a comprehensive view of the direct and indirect effects of RFI, soil pH, and percent sand on soil fauna diversity and SOM pools (Fig. 4.11). Our model explained a substantial portion of the variation in these variables, reinforcing the critical role of regenerative practices in enhancing soil health. The positive influence of RFI on Hill 1 diversity and POM N stocks, and the negative influence on MAOM C saturation deficit, highlight the interconnectedness of soil management, biodiversity, carbon dynamics, and nutrient cycling. While the path analysis refuted the direct effect of soil fauna on C saturation deficit, it is likely that RFI influences both variables independently, with some of the effect of RFI on C saturation occurring indirectly through soil fauna as supported by the negative relationship between Hill 1 diversity and C saturation deficit (Fig. 4.10).

Our study contributes to a growing body of evidence that supports the implementation of regenerative agricultural practices to improve soil health, enhance biodiversity, and mitigate climate change (Giller et al., 2021; Fenster et al., 2021; Prairie et al., 2023; Villat and Nicholas, 2024). Future research should continue to explore these relationships in diverse agroecosystems to develop targeted strategies for sustainable soil management. Understanding the mechanisms by which regenerative systems enhance SOM sequestration and soil biodiversity can inform beneficial stacking of agricultural practices. The interplay between soil fauna and SOM source can also influence the growth of crops and can lead to feedback loops that further shape ecosystem functioning and services (de Vries et al., 2012). Despite their critical importance, the contributions of soil fauna to the success of regenerative systems remains poorly understood and more research is needed to optimize soil biological communities and their beneficial impacts in regenerative agricultural systems. Overall, these findings suggest that regenerative agricultural practices can benefit soil health by enhancing POM and soil fauna diversity, while also helping to sequester atmospheric carbon through increased MAOM storage. This dual benefit emphasizes the potential of regenerative practices to contribute to both agricultural sustainability and climate change mitigation.

## 5. CONCLUSIONS

Our study provides evidence that regenerative agricultural practices can significantly enhance soil fauna diversity and organic matter dynamics in the topsoil. The positive effects of regenerative practices on SOM, POM, and MAOM stocks, along with increased N content, highlight their potential to improve soil health and sequester C. Future research should focus on understanding the long-term impacts of these practices and exploring their applicability across different soil types and climates. By promoting a holistic approach to soil management, regenerative agriculture can play a crucial role in achieving sustainable and resilient agricultural systems.

## REFERENCES

Acosta-Martínez, V., Zobeck, T.M., Allen, V., 2004. Soil microbial, chemical and physical properties in continuous cotton and integrated crop–livestock systems. Soil Science Society of America Journal 68, 1875–1884. doi:10.2136/sssaj2004.1875

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems, in: Paoletti, M.G. (Ed.), Invertebrate Biodiversity as Bioindicators of Sustainable Landscapes. Elsevier, Amsterdam, pp. 19–31. doi:10.1016/B978-0-444-50019-9.50005-4

Alves, L., Veloso, M., Denardin, L., Flores, J., Filippi, D., de Sá, E., Farias, G., Bremm, C., Carvalho, P., Pires, C., Rice, C., Tiecher, T., 2023. Grazing, liming, and fertilization: Shifts on soil fertility and microbial community in a no-till sheep-soybean integrated system. Applied soil ecology 188. doi:10.1016/j.apsoil.2023.104893

Angst, G., Mueller, K.E., Castellano, M.J., Vogel, C., Wiesmeier, M., Mueller, C.W., 2023. Unlocking complex soil systems as carbon sinks: multi-pool management as the key. Nature Communications 14, 2967. doi:10.1038/s41467-023-38700-5

Bailey, V.L., Pries, C.H., Lajtha, K., 2019. What do we know about soil carbon destabilization? Environmental Research Letters 14, 083004. doi:10.1088/1748-9326/ab2c11

Bansal, S., Chakraborty, P., Kumar, S., 2022. Crop–livestock integration enhanced soil aggregate-associated carbon and nitrogen, and phospholipid fatty acid. Scientific Reports 12, 2781. doi:10.1038/s41598-022-06560-6

Bardgett, R.D., van der Putten, W.H., 2014. Belowground biodiversity and ecosystem functioning. Nature 515, 505–511. doi:10.1038/nature13855

Barrios, E., 2007. Soil biota, ecosystem services and land productivity. Ecological Economics, Special Section - Ecosystem Services and Agriculture 64, 269–285. doi:10.1016/j.ecolecon.2007.03.004

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. doi:10.18637/jss.v067.i01

Betancur-Corredor, B., Lang, B., Russell, D.J., 2022. Reducing tillage intensity benefits the soil micro- and mesofauna in a global meta-analysis. European Journal of Soil Science 73, e13321. doi:10.1111/ejss.13321

Bier, R.L., Daniels, M., Oviedo-Vargas, D., Peipoch, M., Price, J.R., Omondi, E., Smith, A., Kan, J., 2024. Agricultural soil microbiomes differentiate in soil profiles with fertility source, tillage, and cover crops. Agriculture, Ecosystems & Environment 368, 109002. doi:10.1016/j.agee.2024.109002

Bowman, M., Poley, K., McFarland, E., 2022. Farmers employ diverse cover crop management strategies to meet soil health goals. Agricultural & Environmental Letters 7, e20070. doi:10.1002/ael2.20070

Brewer, K.M., Gaudin, A.C.M., 2020. Potential of crop-livestock integration to enhance carbon sequestration and agroecosystem functioning in semi-arid croplands. Soil Biology and Biochemistry 149, 107936. doi:10.1016/j.soilbio.2020.107936

Brussaard, L., 1997. Biodiversity and Ecosystem Functioning in Soil. Ambio 26, 563–570.

Brussaard, L., de Ruiter, P.C., Brown, G.G., 2007. Soil biodiversity for agricultural sustainability. Agriculture, Ecosystems & Environment, Biodiversity in Agricultural Landscapes: Investing without Losing Interest 121, 233–244. doi:10.1016/j.agee.2006.12.013

Bu, R., Lu, J., Ren, T., Liu, B., Li, X., Cong, R., 2015. Particulate organic matter affects soil nitrogen mineralization under two crop rotation systems. PLoS ONE 10, e0143835. doi:10.1371/journal.pone.0143835

Córdova-Kreylos, A., Cao, Y., Green, P., Hwang, H., Kuivila, K., LaMontagne, M., Van De Werfhorst, L., Holden, P., Scow, K., 2006. Diversity, composition, and geographical distribution of microbial communities in california salt marsh Sediments. APPLIED AND ENVIRONMENTAL MICROBIOLOGY 72, 3357–3366. doi:10.1128/AEM.72.5.3357-3366.2006

Cotrufo, M.F., Lavallee, J.M., 2022. Chapter One - Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 1–66. doi:10.1016/bs.agron.2021.11.002

Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. Nature Geoscience 12, 989–994. doi:10.1038/s41561-019-0484-6

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988–995. doi:10.1111/gcb.12113

Coulibaly, S.F.M., Aubert, M., Brunet, N., Bureau, F., Legras, M., Chauvat, M., 2022. Short-term dynamic responses of soil properties and soil fauna under contrasting tillage systems. Soil and Tillage Research 215, 105191. doi:10.1016/j.still.2021.105191

Cozim-Melges, F., Ripoll-Bosch, R., Veen, G.F. (Ciska), Oggiano, P., Bianchi, F.J.J.A., van der Putten, W.H., van Zanten, H.H.E., 2024. Farming practices to enhance biodiversity across biomes: a systematic review. Npj Biodiversity 3, 1–11. doi:10.1038/s44185-023-00034-2

Cutler, F. original by L.B. and A., Wiener, R. port by A.L. and M., 2022. randomForest: Breiman and Cutler's Random Forests for Classification and Regression.

Dardonville, M., Bockstaller, C., Villerd, J., Therond, O., 2022. Resilience of agricultural systems: biodiversity-based systems are stable, while intensified ones are resistant and high-yielding. Agricultural Systems 197, 103365. doi:10.1016/j.agsy.2022.103365

de Faccio Carvalho, P.C., Anghinoni, I., de Moraes, A., de Souza, E.D., Sulc, R.M., Lang, C.R., Flores, J.P.C., Terra Lopes, M.L., da Silva, J.L.S., Conte, O., de Lima Wesp, C., Levien, R., Fontaneli, R.S., Bayer, C., 2010. Managing grazing animals to achieve nutrient cycling and soil improvement in no-till integrated systems. Nutrient Cycling in Agroecosystems 88, 259–273. doi:10.1007/s10705-010-9360-x

de Graaff, M.-A., Hornslein, N., Throop, H.L., Kardol, P., van Diepen, L.T.A., 2019. Chapter One - Effects of agricultural intensification on soil biodiversity and implications for ecosystem functioning: A meta-analysis, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 1–44. doi:10.1016/bs.agron.2019.01.001

de Vries, F.T., Liiri, M.E., Bjørnlund, L., Bowker, M.A., Christensen, S., Setälä, H.M., Bardgett, R.D., 2012. Land use alters the resistance and resilience of soil food webs to drought. Nature Climate Change 2, 276–280. doi:10.1038/nclimate1368

Devine, S., Markewitz, D., Hendrix, P., Coleman, D., 2014. Soil Aggregates and associated organic matter under conventional tillage, no-Tillage, and forest succession after three decades. PLOS ONE 9, e84988. doi:10.1371/journal.pone.0084988

Doran, J.W., Stamatiadis, S., Haberern, J., 2002. Soil health as an indicator of sustainable management. Publications from USDA-ARS / UNL Faculty.

Doran, J.W., Zeiss, M.R., 2000. Soil health and sustainability: managing the biotic component of soil quality. Applied Soil Ecology, Special issue: Managing the Biotic component of Soil Quality 15, 3–11. doi:10.1016/S0929-1393(00)00067-6

Drinkwater, L.E., Snapp, S.S., 2007. Nutrients in Agroecosystems: Rethinking the Management Paradigm, in: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, pp. 163–186. doi:10.1016/S0065-2113(04)92003-2

FAO, 2020. Food and Agriculture Organization of the United Nations: FAO soils portal [WWW Document]. URL http://www.fao.org/soils-portal/soil-degradation-restoration/en/ (accessed 1.23.20).

Feng, W., Plante, A.F., Six, J., 2013. Improving estimates of maximal organic carbon stabilization by fine soil particles. Biogeochemistry 112, 81–93. doi:10.1007/s10533-011-9679-7

Fenster, T., Oikawa, P., Lundgren, J., 2021. Regenerative almond production systems improve soil health, biodiversity, and profit. Frontiers in Sustainable Food Systems 5. doi:10.3389/fsufs.2021.664359

Fenster, T.L.D., LaCanne, C.E., Pecenka, J.R., Schmid, R.B., Bredeson, M.M., Busenitz, K.M., Michels, A.M., Welch, K.D., Lundgren, J.G., 2021. Defining and validating regenerative farm systems using a composite of ranked agricultural practices. doi:10.12688/f1000research.28450.1

Georgiou, K., Jackson, R.B., Vindušková, O., Abramoff, R.Z., Ahlström, A., Feng, W., Harden, J.W., Pellegrini, A.F.A., Polley, H.W., Soong, J.L., Riley, W.J., Torn, M.S., 2022. Global stocks and capacity of mineral-associated soil organic carbon. Nature Communications 13, 3797. doi:10.1038/s41467-022-31540-9

Giller, K.E., Hijbeek, R., Andersson, J.A., Sumberg, J., 2021. Regenerative Agriculture: An agronomic perspective. Outlook on Agriculture 50, 13–25. doi:10.1177/0030727021998063

Grace, J.B., Anderson, T.M., Olff, H., Scheiner, S.M., 2010. On the specification of structural equation models for ecological systems. Ecological Monographs 80, 67–87. doi:10.1890/09-0464.1

Haddix, M.L., Paul, E.A., Cotrufo, M.F., 2016. Dual, differential isotope labeling shows the preferential movement of labile plant constituents into mineral-bonded soil organic matter. Global Change Biology 22, 2301–2312. doi:10.1111/gcb.13237

Hansen, P.M., Even, R., King, A.E., Lavallee, J., Schipanski, M., Cotrufo, M.F., 2024. Distinct, direct and climate-mediated environmental controls on global particulate and mineral-associated organic carbon storage. Global Change Biology 30, e17080. doi:10.1111/gcb.17080

Harris, D., Horwáth, W.R., van Kessel, C., 2001. Acid fumigation of soils to remove carbonates prior to total organic carbon or CARBON-13 isotopic analysis. Soil Science Society of America Journal 65, 1853–1856. doi:10.2136/sssaj2001.1853

Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. The ISME Journal 9, 1177–1194. doi:10.1038/ismej.2014.210

Hassink, J., 1997. The capacity of soils to preserve organic C and N by their association with clay and silt particles. Plant and Soil 191, 77–87. doi:10.1023/A:1004213929699

Hassink, J., Whitmore, A.P., 1997. A model of the physical protection of organic matter in soils. Soil Science Society of America Journal 61, 131–139. doi:10.2136/sssaj1997.03615995006100010020x

Hill, M.O., 1973. Diversity and evenness: A unifying notation and its consequences. Ecology 54, 427–432. doi:10.2307/1934352

Hooper, D., Coughlan, J., Mullen, M.R., 2008. Structural Equation Modelling: Guidelines for Determining Model Fit. Electronic Journal of Business Research Methods 6, pp53-60-pp53-60.

Kibblewhite, M.G., Ritz, K., Swift, M.J., 2008. Soil health in agricultural systems. Philosophical Transactions of the Royal Society B: Biological Sciences 363, 685–701. doi:10.1098/rstb.2007.2178

King, A.E., Amsili, J.P., Córdova, S.C., Culman, S., Fonte, S.J., Kotcon, J., Masters, M.D., McVay, K., Olk, D.C., Prairie, A.M., Schipanski, M., Schneider, S.K., Stewart, C.E., Cotrufo, M.F., 2024. Constraints on mineral-associated and particulate organic carbon response to regenerative management: carbon inputs and saturation deficit. Soil and Tillage Research 238, 106008. doi:10.1016/j.still.2024.106008

Knebl, L., Leithold, G., Schulz, F., Brock, C., 2017. The role of soil depth in the evaluation of management-induced effects on soil organic matter. European Journal of Soil Science 68, 979–987. doi:10.1111/ejss.12492

Koenker, R., Portnoy, S., Tian Ng, P., Melly, B., Zeileis, A.Z. (Contributions to dynrq code essentially identical to his dynlm, Grosjean, P., routines), C., Saad, Y., Chernozhukov, V., Fernandez-Val, I., Ripley, B.D., 2024. quantreg: Quantile Regression.

Lange, M., Eisenhauer, N., Sierra, C.A., Bessler, H., Engels, C., Griffiths, R.I., Mellado-Vázquez, P.G., Malik, A.A., Roy, J., Scheu, S., Steinbeiss, S., Thomson, B.C., Trumbore, S.E., Gleixner, G., 2015. Plant diversity increases soil microbial activity and soil carbon storage. Nature Communications 6, 6707. doi:10.1038/ncomms7707

Lavallee, J.M., Soong, J.L., Cotrufo, M.F., 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. Global Change Biology 26, 261–273. doi:10.1111/gcb.14859

Lavelle, P., 1997. Faunal activities and soil processes: Adaptive strategies that determine ecosystem function, in: Begon, M., Fitter, A.H. (Eds.), Advances in Ecological Research. Academic Press, pp. 93–132. doi:10.1016/S0065-2504(08)60007-0

Lavelle, P., Decaëns, T., Aubert, M., Barot, S., Blouin, M., Bureau, F., Margerie, P., Mora, P., Rossi, J.-P., 2006. Soil invertebrates and ecosystem services. European Journal of Soil Biology, ICSZ 42, S3–S15. doi:10.1016/j.ejsobi.2006.10.002

Lavelle, P., Spain, A., Fonte, S., Bedano, J.C., Blanchart, E., Galindo, V., Grimaldi, M., Jimenez, J.J., Velasquez, E., Zangerlé, A., 2020. Soil aggregation, ecosystem engineers and the C cycle. Acta Oecologica 105, 103561. doi:10.1016/j.actao.2020.103561

Lehman, R.M., Cambardella, C.A., Stott, D.E., Acosta-Martinez, V., Manter, D.K., Buyer, J.S., Maul, J.E., Smith, J.L., Collins, H.P., Halvorson, J.J., Kremer, R.J., Lundgren, J.G., Ducey, T.F., Jin, V.L., Karlen, D.L., 2015. Understanding and enhancing soil biological health: The solution for reversing soil degradation. Sustainability 7, 988–1027. doi:10.3390/su7010988

Lehman, R.M., Osborne, S.L., McGraw, K., 2019. Stacking agricultural management tactics to promote improvements in soil structure and microbial activities. Agronomy 9, 539. doi:10.3390/agronomy9090539

Lemaire, G., Franzluebbers, A., Carvalho, P.C. de F., Dedieu, B., 2014. Integrated crop–livestock systems: Strategies to achieve synergy between agricultural production and environmental quality. Agriculture, Ecosystems & Environment, Integrated Crop-Livestock System Impacts on Environmental Processes 190, 4–8. doi:10.1016/j.agee.2013.08.009

Leuthold, S.J., Haddix, M.L., Lavallee, J., Cotrufo, M.F., 2022. Physical fractionation techniques, in: Reference Module in Earth Systems and Environmental Sciences. Elsevier. doi:10.1016/B978-0-12-822974-3.00067-7

Liebman, M., Schulte, L.A., 2015. Enhancing agroecosystem performance and resilience through increased diversification of landscapes and cropping systems. Elementa: Science of the Anthropocene 3, 000041. doi:10.12952/journal.elementa.000041

Liu, X., Herbert, S.J., Hashemi, A.M., Zhang, X., Ding, G., 2018. Effects of agricultural management on soil organic matter and carbon transformation - a review. doi:10.17221/3544-pse

Lori, M., Symnaczik, S., Mäder, P., Deyn, G.D., Gattinger, A., 2017. Organic farming enhances soil microbial abundance and activity—A meta-analysis and meta-regression. PLOS ONE 12, e0180442. doi:10.1371/journal.pone.0180442

Lugato, E., Lavallee, J.M., Haddix, M.L., Panagos, P., Cotrufo, M.F., 2021. Different climate sensitivity of particulate and mineral-associated soil organic matter. Nature Geoscience 14, 295–300. doi:10.1038/s41561-021-00744-x

Mao, H.-R., Cotrufo, M.F., Hart, S.C., Sullivan, B.W., Zhu, X., Zhang, J., Liang, C., Zhu, M., 2024. Dual role of silt and clay in the formation and accrual of stabilized soil organic carbon. Soil Biology and Biochemistry 192, 109390. doi:10.1016/j.soilbio.2024.109390

Martin, E.A., Feit, B., Requier, F., Friberg, H., Jonsson, M., 2019. Chapter Three - Assessing the resilience of biodiversity-driven functions in agroecosystems under environmental change, in: Bohan, D.A., Dumbrell, A.J. (Eds.), Advances in Ecological Research, Resilience in Complex Socio-Ecological Systems. Academic Press, pp. 59–123. doi:10.1016/bs.aecr.2019.02.003

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2014. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecological Applications 24, 560–570. doi:10.1890/13-0616.1

Meena, R.S., Singh, A.K., Jatav, S.S., Rai, S., Pradhan, G., Kumar, S., Mina, K.K., Jhariya, M.K., 2024. Chapter 10 - Significance of soil organic carbon for regenerative agriculture and ecosystem services, in: Singh, K., Ribeiro, M.C., Calicioglu, Ö. (Eds.), Biodiversity and Bioeconomy. Elsevier, pp. 217–240. doi:10.1016/B978-0-323-95482-2.00010-9

Merckx, R., Diels, J., Vanlauwe, B., Sanginga, N., Denef, K., Oorts, K., 2001. Soil Organic Matter and Soil Fertility, in: Sustaining Soil Fertility in West Africa. John Wiley & Sons, Ltd, pp. 69–89. doi:10.2136/sssaspecpub58.ch4

Minasny, B., McBratney, A.B., 2006. A conditioned Latin hypercube method for sampling in the presence of ancillary information. Computers & Geosciences 32, 1378–1388. doi:10.1016/j.cageo.2005.12.009

Morugán-Coronado, A., Pérez-Rodríguez, P., Insolia, E., Soto-Gómez, D., Fernández-Calviño, D., Zornoza, R., 2022. The impact of crop diversification, tillage and fertilization type on soil total microbial, fungal and bacterial abundance: A worldwide meta-analysis of agricultural sites. Agriculture, Ecosystems & Environment 329, 107867. doi:10.1016/j.agee.2022.107867

Narayana, N., Kingery, W., Shankle, M., Shanmugam, S., 2022. Differential response of soil microbial diversity and community composition influenced by cover crops and fertilizer treatments in a dryland soybean production system. AGRONOMY-BASEL 12. doi:10.3390/agronomy12030618

Nardo, M., Saisana, M., Saltelli, A., Tarantola, S., Hoffmann, A., Giovannini, E., 2008. Handbook on Constructing Composite Indicators: Methodology and User Guide [WWW Document]. JRC Publications Repository. URL https://publications.jrc.ec.europa.eu/repository/handle/JRC47008 (accessed 6.5.24).

Nunes, M.R., van Es, H.M., Schindelbeck, R., Ristow, A.J., Ryan, M., 2018. No-till and cropping system diversification improve soil health and crop yield. Geoderma 328, 30–43. doi:10.1016/j.geoderma.2018.04.031

Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M.D., Durand, S., Evangelista, H.B.A., FitzJohn, R., Friendly, M., Furneaux, B., Hannigan, G., Hill, M.O., Lahti, L., McGlinn, D., Ouellette, M.-H., Cunha, E.R., Smith, T., Stier, A., Braak, C.J.F.T., Weedon, J., 2024. vegan: Community Ecology Package.

Parker, S.S., 2010. Buried treasure: soil biodiversity and conservation. Biodiversity and Conservation 19, 3743–3756. doi:10.1007/s10531-010-9924-8

Paudel, D., Wang, L., Poudel, R., Acharya, J., Victores, S., de Souza, C., Rios, E., Wang, J., 2023. Elucidating the effects of organic vs. conventional cropping practice and rhizobia inoculation on rhizosphere microbial diversity and yield of peanut. ENVIRONMENTAL MICROBIOME 18. doi:10.1186/s40793-023-00517-6

Pecenka, J.R., Lundgren, J.G., 2018. The importance of dung beetles and arthropod communities on degradation of cattle dung pats in eastern South Dakota. PeerJ 6, e5220. doi:10.7717/peerj.5220

Piñeiro, G., Paruelo, J.M., Jobbágy, E.G., Jackson, R.B., Oesterheld, M., 2009. Grazing effects on belowground C and N stocks along a network of cattle exclosures in temperate and subtropical grasslands of South America. Global Biogeochemical Cycles 23. doi:10.1029/2007GB003168

Poeplau, C., Don, A., Six, J., Kaiser, M., Benbi, D., Chenu, C., Cotrufo, M.F., Derrien, D., Gioacchini, P., Grand, S., Gregorich, E., Griepentrog, M., Gunina, A., Haddix, M., Kuzyakov, Y., Kühnel, A., Macdonald, L.M., Soong, J., Trigalet, S., Vermeire, M.-L., Rovira, P., van Wesemael, B., Wiesmeier, M., Yeasmin, S., Yevdokimov, I., Nieder, R., 2018. Isolating organic carbon fractions with varying turnover rates in temperate agricultural soils – A comprehensive method comparison. Soil Biology and Biochemistry 125, 10–26. doi:10.1016/j.soilbio.2018.06.025

Prairie, A.M., King, A.E., Cotrufo, M.F., 2023. Restoring particulate and mineral-associated organic carbon through regenerative agriculture. Proceedings of the National Academy of Sciences 120, e2217481120. doi:10.1073/pnas.2217481120

Puissant, J., Villenave, C., Chauvin, C., Plassard, C., Blanchart, E., Trap, J., 2021. Quantification of the global impact of agricultural practices on soil nematodes: A meta-analysis. Soil Biology and Biochemistry 161, 108383. doi:10.1016/j.soilbio.2021.108383

Rayne, N., Aula, L., 2020. Livestock manure and the impacts on soil health: A review. Soil Systems 4, 64. doi:10.3390/soilsystems4040064

Rehberger, E., West, P., Spillane, C., McKeown, P., 2023. What climate and environmental benefits of regenerative agriculture practices? an evidence review. ENVIRONMENTAL RESEARCH COMMUNICATIONS 5. doi:10.1088/2515-7620/acd6dc

Rizk, M.A., Mikhail, W.Z.A., 1999. Impact of no-tillage agriculture on soil fauna diversity. Zoology in the Middle East 18, 113–120. doi:10.1080/09397140.1999.10637787

Rosenzweig, S.T., Fonte, S.J., Schipanski, M.E., 2018. Intensifying rotations increases soil carbon, fungi, and aggregation in semi-arid agroecosystems. Agriculture, Ecosystems & Environment 258, 14–22. doi:10.1016/j.agee.2018.01.016

Rosseel, Y., 2012. lavaan: An R Package for Structural Equation Modeling. Journal of Statistical Software 48, 1–36. doi:10.18637/jss.v048.i02

Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting Soil pH Effects on Fungal and Bacterial Growth Suggest Functional Redundancy in Carbon Mineralization. Applied and Environmental Microbiology 75, 1589–1596. doi:10.1128/AEM.02775-08

Sanderman, J., Hengl, T., Fiske, G.J., 2017. Soil carbon debt of 12,000 years of human land use. Proceedings of the National Academy of Sciences 114, 9575–9580. doi:10.1073/pnas.1706103114

Schmid, R.B., Welch, K.D., Teague, R., Lundgren, J.G., 2024. Adaptive multipaddock (AMP) pasture management increases arthropod community guild diversity without increasing pests. Rangeland Ecology & Management 94, 141–148. doi:10.1016/j.rama.2024.03.001

Shannon, C.E., 1948. A mathematical theory of communication. The Bell System Technical Journal 27, 379–423. doi:10.1002/j.1538-7305.1948.tb01338.x

Shipley, B., 2016. Cause and Correlation in Biology: A User's Guide to Path Analysis, Structural Equations and Causal Inference with R, 2nd ed. Cambridge University Press, Cambridge. doi:10.1017/CBO9781139979573

Singh, R.K., Murty, H.R., Gupta, S.K., Dikshit, A.K., 2009. An overview of sustainability assessment methodologies. Ecological Indicators 9, 189–212. doi:10.1016/j.ecolind.2008.05.011

Six, J., Elliott, E.T., Paustian, K., 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Science Society of America Journal 63, 1350–1358. doi:10.2136/sssaj1999.6351350x

Smith, P., Cotrufo, M.F., Rumpel, C., Paustian, K., Kuikman, P.J., Elliott, J.A., McDowell, R., Griffiths, R.I., Asakawa, S., Bustamante, M., House, J.I., Sobocká, J., Harper, R., Pan, G., West, P.C., Gerber, J.S., Clark, J.M., Adhya, T., Scholes, R.J., Scholes, M.C., 2015. Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils. SOIL 1, 665–685. doi:https://doi.org/10.5194/soil-1-665-2015

Sokol, N.W., Kuebbing, S.E., Karlsen-Ayala, E., Bradford, M.A., 2019a. Evidence for the primacy of living root inputs, not root or shoot litter, in forming soil organic carbon. The New Phytologist 221, 233–246. doi:10.1111/nph.15361

Sokol, N.W., Sanderman, J., Bradford, M.A., 2019b. Pathways of mineral-associated soil organic matter formation: Integrating the role of plant carbon source, chemistry, and point of entry. Global Change Biology 25, 12–24. doi:10.1111/gcb.14482

Stanley, P.L., Wilson, C., Patterson, E., Machmuller, M.B., Cotrufo, M.F., 2024. Ruminating on soil carbon: Applying current understanding to inform grazing management. Global Change Biology 30, e17223. doi:10.1111/gcb.17223

Swift, M.J., Izac, A.-M.N., van Noordwijk, M., 2004. Biodiversity and ecosystem services in agricultural landscapes—are we asking the right questions? Agriculture, Ecosystems & Environment, Environmental Services and Land Use Change: Bridging the Gap between Policy and Research in Southeast Asia 104, 113–134. doi:10.1016/j.agee.2004.01.013

Talukder, B., W. Hipel, K., W. vanLoon, G., 2017. Developing composite indicators for agricultural sustainability assessment: Effect of normalization and aggregation techniques. Resources 6, 66. doi:10.3390/resources6040066

Tian, L., Dell, E., Shi, W., 2010. Chemical composition of dissolved organic matter in agroecosystems: Correlations with soil enzyme activity and carbon and nitrogen mineralization. Applied Soil Ecology 46, 426–435. doi:10.1016/j.apsoil.2010.09.007

Tilman, D., Balzer, C., Hill, J., Befort, B.L., 2011. Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences 108, 20260–20264. doi:10.1073/pnas.1116437108

van Capelle, C., Schrader, S., Brunotte, J., 2012. Tillage-induced changes in the functional diversity of soil biota – A review with a focus on German data. European Journal of Soil Biology 50, 165–181. doi:10.1016/j.ejsobi.2012.02.005

van der Pol, L.K., Robertson, A., Schipanski, M., Calderon, F.J., Wallenstein, M.D., Cotrufo, M.F., 2022. Addressing the soil carbon dilemma: Legumes in intensified rotations regenerate soil carbon while maintaining yields in semi-arid dryland wheat farms. Agriculture, Ecosystems & Environment 330, 107906. doi:10.1016/j.agee.2022.107906

Venter, Z.S., Jacobs, K., Hawkins, H.-J., 2016. The impact of crop rotation on soil microbial diversity: A meta-analysis. Pedobiologia 59, 215–223. doi:10.1016/j.pedobi.2016.04.001

Villat, J., Nicholas, K.A., 2024. Quantifying soil carbon sequestration from regenerative agricultural practices in crops and vineyards. Frontiers in Sustainable Food Systems 7. doi:10.3389/fsufs.2023.1234108

Wickham, H., François, R., Henry, L., Müller, K., RStudio, 2019. dplyr: A grammar of data manipulation.

Yu, W., Huang, W., Weintraub-Leff, S.R., Hall, S.J., 2022. Where and why do particulate organic matter (POM) and mineral-associated organic matter (MAOM) differ among diverse soils? Soil Biology and Biochemistry 172, 108756. doi:10.1016/j.soilbio.2022.108756

# CHAPTER 5: PREDATORY PRESSURE DRIVES BACTERIVORE-STIMULATED MICROBIAL NECROMASS ACCUMULATION AND MAOM FORMATION[10]

## 1. INTRODUCTION

Soil organic matter (SOM) is pivotal for soil fertility and carbon (C) sequestration; however, SOM is incredibly complex and requires separation into multiple components with contrasting behavior to study and predict its dynamics. Mineral-associated organic matter (MAOM) forms early in decomposition when soluble plant inputs bind to minerals directly, or after being transformed by soil microbes, while particulate organic matter (POM) forms as a result of partial decomposition and physical fragmentation of the structural plant components (Cotrufo et al., 2015; Fulton-Smith and Cotrufo, 2019; Haddix et al., 2020). In mineral soils, when not protected in microaggregates, POM represents a transient C pool, rapidly cycling and susceptible to environmental changes that influence decomposition, whereas MAOM consists of more stable organo-mineral complexes that can persist for decades to millennia, playing a vital role in long-term C storage (Poeplau et al., 2018; Lavallee et al., 2020; Hansen et al., 2024). This distinction helps in uncovering the mechanisms of SOM formation and persistence and allows us to better predict soil C dynamics under varying environmental and management scenarios.

Understanding the biological mechanisms controlling the formation and persistence of these SOM fractions is particularly important for managing soil health and developing strategies to mitigate climate change (Lehman et al., 2015). It is now widely recognized that SOM stability is largely governed by the anabolic activities of microorganisms. This understanding underscores the concept that the most persistent organic C in soil is in large part not derived directly from plant litter or its residues, but rather from C that has been processed through microbial activity (Liang and Balser, 2011; Miltner et al., 2012; Cotrufo et al.,

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[10] Manuscript in preparation for peer review publication in Soil Biology and Biochemsitry with André Franco, Cecilia Milano de Tomasel, Chao Liang, and M. Francesca Cotrufo

2013). Both theoretical and empirical studies support the significant contribution of microorganisms to the persistent MAOM pool, through continuous cycles of cell generation, population growth, death, decay, and necromass formation. Microbial necromass, the remains of dead microbial biomass, is now recognized as a significant contributor to MAOM formation, comprising 24-60% of total SOM globally (Liang et al., 2019, 2020; Zhu et al., 2020; Deng and Liang, 2022). The production and stabilization of microbial necromass, along with the associated microbial traits, are crucial for understanding its persistence in SOM (Buckeridge et al., 2020; Kästner et al., 2021; Camenzind et al., 2023; Angst et al., 2024; Peng et al., 2024).

Despite considerable progress in understanding the microbial contributions to SOM dynamics, the role of soil fauna remains less explored. Soil fauna are recognized for their role in accelerating decomposition and influencing POM dynamics through physical and biological mechanisms such as litter fragmentation, bioturbation, and gut passage (Wickings and Grandy, 2011; Angst et al., 2017; Frouz, 2018). However, their influence on the stabilization of MAOM by affecting microbial turnvover and necromass formation, particularly through direct and indirect interactions with microbes, remains underexplored (Grandy et al., 2016; Soong and Nielsen, 2016; Thakur and Geisen, 2019). Studies that quantify necromass production and recycling often fail to consider the potential of soil fauna to stimulate microbial turnover and necromass formation (Grandy et al., 2016).

Direct feeding interactions between fungi and fungivores, or between bacteria and bacterivores, can enhance C and nutrient fluxes (Crowther et al., 2015; Soong et al., 2016). This suggests that higher trophic level soil fauna can influence soil C and nutrient cycling through predation within the soil food web (Sackett et al., 2010; Grandy et al., 2016; Kästner and Miltner, 2018). Microbial grazing, particularly by bacterivorous nematodes, has been shown to affect microbial community structure and function, leading to altered microbial turnover and activity (Djigal et al., 2004; Trap et al., 2016; Jiang et al., 2017; Erktan et al., 2020). These bacterivores can release C and N bound in senesced cells, contributing to maintenance of younger bacteria cells and driving turnover (Trap et al., 2016). Moreover, because bacterivores must maintain stoichiometric homeostasis, much of the ingested N is released, increasing N availability and promoting bacterial growth (Ferris et al., 1998; Sinsabaugh et al., 2013). However, the magnitude and direction of the effects of bacterivores on microbial populations are not always consistent and the overarching effects of such interactions on SOM dynamics remains unclear. This is particularly true concerning the impact of higher trophic levels, such as mesostigmatid mites, which can further influence these dynamics by modifying bacterial predator-prey relationships (Azevedo et al., 2020; Lucas et al., 2020).

The complexity of soil ecosystems necessitates sophisticated techniques to unravel the interactions within. Employing stable isotopes combined with soil microcosm experiments offers a novel approach to trace C and nitrogen (N) flows, particularly focusing on the impacts of soil fauna—specifically the roles of bacterial feeding nematodes and predatory mites—on microbial dynamics. Our primary objective was to delve into the role of soil fauna as drivers of microbial necromass production and SOM persistence through their effects on microbial growth, activity, and turnover. To uncover these effects, we conducted a soil microcosm experiment to test the following hypotheses: (1) The presence of bacterivorous nematodes will reduce microbial biomass but stimulate soil microbial activity and microbial turnover leading to greater necromass production and subsequent MAOM formation; (2) The introduction of higher trophic level predators will alleviate grazing pressure on bacteria, resulting in less necromass production and reduced MAOM formation. Our alternative hypothesis 2 is that the addition of a mite predator will alter nematode behavior and maintain nematode populations at intermediate densities resulting in optimal bacterial growth, turnover, and necromass formation resulting in greater MAOM formation (Lucas et al., 2020). This research provides new insights into the interplay between soil fauna and microbial dynamics, advancing our understanding of SOM stabilization and ecosystem processes.

## 2. MATERIALS AND METHODS

### *2.1 Soil used in the experiment and defaunation pretreatment*

For the incubation experiment, soil was sourced from the USDA Central Great Plains Research site, Akron, CO (40.16 N, 103.14 W). Soil was clay loam (42.6% sand, 16.2% silt, 41.2% clay) with 1.80% organic carbon (OC) and 0.19% nitrogen (N) and a pH of 7.3. There was no evidence of carbonates after fizz test, and the soil natural abundance $\delta^{13}C$ was -19.8‰. To remove native soil fauna, prior to incubation soil was thermally treated following Franco et al. (2017). Briefly, sieved soil (6.25 mm) was placed into aluminum dishes with small holes at the bottom, ensuring a depth of 5 cm. Soil was then pre-wetted by gently spraying the surface until water passed through the holes in the bottom of the trays. The pre-wet soil was left at 4 °C for 24 h. Next, the trays were transferred to an oven set at 65 °C for 48 h. Thermal treatment kills 99% of fauna while avoiding major disturbances to microbial communities. To ensure the absence of native fauna, we used six 100 g subsamples of defaunated soil for nematode extraction using Baermann funnels (Hooper, 1970). Nematodes in soil subsamples were extracted daily for three days, stored at 4 °C, and counted within three days of extraction using an inverted microscope (Olympus CKX41, 200X magnification). The soil underwent additional thermal treatment until acceptable native fauna levels were reached, i.e., with no bacterial-feeding nematodes and fewer than three fungal-feeding nematodes per 100g of soil.

### *2.2 Production and defaunation of $^{13}C$ and $^{15}N$ enriched plant litter*

Prior to experiment setup, Blue grama (*Bouteloua gracilis*) was grown from rhizomes in soil-free potting mix for one growing season in a continuous labeling chamber at 4 atom% $^{13}C$-$CO_2$ atmosphere, fertilized weekly for 21 weeks with a 7 atom% $^{15}N$-$KNO_3$ solution (Soong et al., 2014). After the growing season, plants were harvested and roots were separated from shoots. Shoots were then washed, air-dried, and a subsample analyzed for C (37.1%), N (1.3%), $^{13}C$ ($d^{13}C$ =1406‰; 2.57 $^{13}C$ atom%) and $^{15}N$ ($\delta^{15}N$ 18896‰; 6.8 $^{15}N$ atom%) by vario ISOTOPE CUBE elemental analyzer (EA; Elementar Langenselbold, Germany) coupled to an isoprime precisION Isotope Ratio Mass Spectrometer (IRMS; Elementar UK

Manchester, United Kingdom). Prior to incubation, the labeled litter was defaunated in aliquotes of 10 g as described for soil above.

### *2.3 Soil fauna isolation and incubation*

A diverse community of bacterivorous nematodes isolated from Lory State Park grassland soil was obtained from cultures grown by the Wall lab at Colorado State University and used for the incubation. Briefly, 100 g of soil nematodes were extracted using the Baermann funnel technique for 3 days, then from the 5 ml sample bacteria feeding nematodes were handpicked under a dissecting scope with an eyelash (superfine eyelash with handle, Ted Pella, Inc., prod no. 113). Nematodes were then added to a petri dish containing Nematode Growth Medium (NGM) for nematode growth and stored at 4°C, until used for the incubation.

Mites (*Stratiolaelaps scimitus*) were obtained from a commercial biocontrol supplier (ARBICO Organics). These generalist predators of the order Mesostigmata are widely used to control nematodes and other invertebrate pests in soil (Azevedo et al., 2020; Yang et al., 2020).

### *2.4 Design and establishment of the incubation experiment*

We tested our hypotheses in a 6-month laboratory microcosm incubation experiment. A total of 60 microcosms (~946ml glass jars) containing 100g dry soil were incubated in a constant temperature room (25°C) in the Soil Innovation Laboratory at Colorado State University. We applied three treatments in a completely randomized design: 1) ‘nem’: inoculum with bacterivorous nematodes and isotopically labeled litter (produced as described below); 2) ‘mite’: inoculum with bacterivorous nematodes + predatory mesostigmatid mites and labeled litter; 3) ‘no-fauna’: control with labeled litter only. Each treatment incubation had 6 replicates for each of the 3 harvests (1 mo, 3 mo, 6 mo) for a total of 18 microcosms per treatment. At microcosm establishment,100 g of defaunated soil was thoroughly mixed with 1 g of finely chopped (~1 cm) enriched shoot litter and combined in plastic cups (~237ml) and kept at 4 °C until the fauna were ready to be introduced.

For the nematode addition, NGM media containing bacterial feeding nematodes were extracted as described above. Solution containing extracted nematodes were counted as above and a aliquot containing ~300 nematodes (0.2 ml) was added to each of 36 microcosms (i.e., "nem" and "mite" treatments) based on averages found in similar soils (van den Hoogen et al., 2020).

Fifteen mites were handpicked using a fine paintbrush and individually added to 18 (i.e., "mite" treatment) of the 54 microcosms. To ensure the establishment of mite colonies, the remaining mites were cultured and 5 mites were reintroduced to the same microcosms at intervals of 15, 30, and 45 days from the onset of incubation following Laakso and Setälä (1999) and Azevedo et al. (2020). Mite numbers were chosen to be consistent with average densities of Mesostigmata mites in grassland soils (Coleman et al., 2004). To prevent escape and contamination between treatments, the outer rims of the cups were coated with a thin layer of petroleum jelly.

Additionally, 6 natural abundance controls, without fauna inocula or enriched litter, were established with 100 g of defaunated soil to measure the end-member isotopic composition of native SOM and soil respiration. All microcosms were incubated in the dark 50% water filled pore space (WFPS). Optimal moisture at 50% WFPS by weight was achieved through regular re-weighing and bi-weekly water addition. Mass of water necessary to achieve WFPS of 0.5 was calculated as follows:

$$W = \frac{0.5\Phi}{BD} \times ODwt \tag{1}$$

where $W$ is grams of water to be added, $ODwt$ is oven-dry weight of soil (100g), $BD$ is bulk density measured (1.087 g/cm$^3$), and $\Phi$ is porosity calculated as:

$$\Phi = 1 - BD/D_P \tag{2}$$

where $D_P$ is particle density estimated to be 2.65 g/cm$^3$.

### *2.5 Soil and labeled litter respiration*

We estimated microbial catabolic activity by measuring the amount and $^{13}C$ enrichment of the $CO_2$ produced by the mineralization of the SOM and the labeled litter regularly throughout the incubation, on the subset of microcosms harvested at 6 months (18) and control microcosms (6). Soil-filled cups were placed inside 32-oz mason jars, with 20 ml added to the bottom of the jar to maintain humidity. Soil respiration was measured on day 0, 3, 5, 7, 9, 12, 14, 17, 20, 24, 27, 31 34, 39, 45, 52, 62, 69, 77, 88, 101, 111, 123, 131, 140, 154, 167, and 182 of the incubation by determining the $CO_2$ concentration accumulated in the jar headspace during the time elapsed since the last sampling. After mixing the jar's headspace, a 1-ml subsample was manually injected into an infrared gas analyzer (IRGA, model LI6252, LICOR). Calibration with an external standard curve determined $CO_2$ concentration. On alternate sampling days, a 20 ml subsample was taken from the headspace of each jar and injected into a 12 ml airtight vial for subsequent analysis of $^{13}C$-$CO_2$ using an IsoFLOW GHG (Elementar Langenselbold, Germany) coupled to an IRMS. After headspace was measured, jars were flushed for 1 min with $CO_2$ free air, and measured again for concentration. This was subtracted from the subsequent measurement at the following timepoint. The final respiration rate is expressed on a dry soil basis as µg $CO_2$-C g soil$^{-1}$ day$^{-1}$. The respiration rate was converted to cumulative respiration by summing the rates at each time point. The respiration data and isotopic signatures provided in the data product were used to calculate the amount of $CO_2$-C coming from the added litter with a mixing model, as described below.

### *2.6 Microcosm harvest and fauna analyses*

During each of the three harvests at 1, 3, and 6 months, a total of 18 microcosms were sampled. Additionally, the 6 control microcosms were harvested at 6 months. At harvests litter could not be isolated from soils, and therefore was considered part of the soil organic matter. Soils were carefully removed from each incubation cup and homogenized using a 2 mm sieve, and then subsampled for following analyses. For amino sugar analysis and DNA extraction, 6 g of soil was frozen at -80°C. To determine microbial biomass, 20 g of fresh soil was utilized. For nematode extraction, 50 g of fresh soil was required. Additionally, 24 g of soil was designated for fractionation and mite extraction, with at least 10g reserved for the fractionation process. Data from the 3-month harvest was excluded from further analyses due to funding and time constraints.

Nematodes in soil subsamples were extracted using Baermann funnels (Hooper, 1970), with daily removals over three days, and then stored at 4°C. Within three days of extraction, nematodes were counted using an inverted microscope. Subsequently, 75 individuals were meticulously handpicked using an eyelash under a dissecting microscope and transferred to pre-weighed tin capsules (8x5 mm, Elemental Microanalysis BN/170056) containing 120 µL of deionized water. The tin capsules were desiccated for three days, re-weighed to obtain final sample weights, and then analyzed for %C, %N, $^{13}C$ and $^{15}N$ using an EA-IRMS, as described above for the litter. We attempted to extract mites using a Berlese-Tullgren funnels (48 h dark followed by 48 h low, 48 h medium and 48 h full light/heat intensity) (Crossley and Blair, 1991), but did not successfully recover any individuals. This likely resulted from the reduced soil quantity (24 g) available for extraction, compared to the typical 100-500 g used in standard Tullgren funnel procedures.

### *2.7 Microbial biomass*

Total microbial biomass C and N was estimated using the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, soil samples were divided into “control” and “fumigated” groups. Control soils were immediately extracted with 0.05 M $K_2SO_4$ by adding 50 mL to 10 g fresh soil and shaken on a rotary shaker at 200 rpm for 2 hr. Extracts were filtered through a Whatman 40 filter (8 µm). Fumigated samples were exposed to chloroform in a vacuum chamber, left to fumigate for 24 hours, evacuated for 4 hours, and then processed identically to control samples. All extracts were frozen at −20 °C, lyophilized (freeze-dried), and the resulting residues analyzed for isotopic composition. Three blanks containing only $K_2SO_4$ were included with each group and concentrations subtracted from sample concentrations.

As the same soil was used across all samples, to avoid artifacts of applying an unverified extraction efficiency for our soil, we used the difference in C or N content between fumigated and control (unfumigated) samples as a proxy for microbial biomass C (MBC) and N (MBN), respectively.

### *2.8 Amino sugar analysis*

To estimate microbial necromass C and N, amino sugars were extracted from bulk soil following a protocol modified from Liang et al. (2012). Briefly, subsamples of soil containing approximately 0.3 mg of N were weighed into acid-digestion vessels and boiled (at 105 °C) with concentrated (6 M) hydrochloric acid for 8 h. The hydrolysates were purified through several steps. First, samples were filtered through fiber filters (GF/A; 90 mm) and dried using a rotary evaporator at 45 °C under vacuum. The residue was dissolved in deionized water, neutralized, and centrifuged to remove salts. The supernatant was freeze-dried, resuspended in methanol, dried using N gas, dissolved in 1 ml deionized water, and then lyophilized. For derivatization, the sample was heated with hydroxylamine hydrochloride and 4-(dimethylamino) pyridine in a pyridine-methanol mixture, followed by acetic anhydrite at 75–80 °C. Dichloromethane was added, and the excess reagent was removed through washing with hydrochloric acid and deionized water. The sample was then dried and resuspended in an ethyl acetate-hexane mixture (1:1) for analysis. Myo-inositol and methylglucamine were used as internal and recovery standards. The derivatized extracts were measured on a gas chromatograph coupled to a mass spectrometer (GC-MS, Thermo Scientific TSQ 8000 Evo Triple Quadrupole), and glucosamine (GluN), galactosamine (GalN), and muramic acid (MurA) quantified against an external standard of known concentrations containing the target analytes. Bacterial necromass C was calculated using a conversion factor of 45 for MurA, while fungal necromass C was calculated by subtracting the bacterial GluN content and applying a conversion factor of 9 for the corrected GluN (Liang et al., 2019). Bacterial necromass N was calculated using a conversion factor of 11 for MurA, and fungal necromass N was calculated by subtracting bacterial GluN and applying a conversion factor of 0.9 (Deng and Liang, 2022):

$$Bacterial\ necromass\ C = MurA * 45$$

( 3 )

$$Fungal\ necromass\ C = \left(\frac{GluN}{179.17} - 2 * \frac{MurA}{251.23}\right) * 179.17 * 9$$

( 4 )

$$Bacterial\ necromass\ N = MurA * 11$$

( 5 )

$$Fungal\ necromass\ C = \left(\frac{GluN}{179.17} - 2 * \frac{MurA}{251.23}\right) * 179.17 * 0.9$$

( 6 )

Where 179.17 is the molecular weight of GluN and 251.23 is the molecular weight of MurA. We then quantified the relative contributions of bacterial and fungal necromass C and N to total SOC and SON, respectively.

### *2.9 SOM fractionation*

A representative subsample from each microcosm was fractionated by size and density to obtain POM, MAOM, and CHAOM fraction (Leuthold et al., 2022). A combined size and density fractionation scheme was chosen for its ability to isolate the most chemically and functionally discrete pools of SOM (Leuthold et al., 2024). In brief, after mechanical dispersion in sodium polytungstate (SPT), SOM was separated into a light fraction (POM < 1.85 g cm$^{-3}$) and a heavy fraction. The latter was separated through wet sieving by size, into a sand-sized (CHAOM >53 µm) and a silt-and-clay fraction (MAOM < 53 µm). Fractionation was accepted when fractions mass recovery was ±5% of the bulk soil mass. It should be noted that any remaining partly decomposed litter fragment would be isolated as POM. Bulk soils and all fractions were analyzed for %C, %N, $\delta^{13}$C and $\delta^{15}$N using an EA-IRMS, as described above. Fractions C and N recoveries were 83.6 ± 1.5%, and 83.1 ± 1.3%, respectively. For the purposes of this dissertation, we will focus exclusively on findings related to POM and MAOM.

### *2.10 Data analyses*

The litter C (and N) contribution to the $CO_2$, bulk soil, microbial biomass, amino sugars and, SOM fractions, was assessed using the isotopic mixing model:

$$f_{litter} = \frac{\delta_S - \delta_B}{\delta_{litter} - \delta_B} \tag{7}$$

where $f_{litter}$ is the fraction of litter-derived C (or N) contributing to $CO_2$, microbial biomass, bulk soil, and SOM fractions. $\delta_S$ and $\delta_B$ are the $\delta^{13}C$ (or $\delta^{15}N$) of the specific $CO_2$, microbial biomass, bulk soil, or SOM sample from the treatment ($\delta_S$) and the natural abundance control soil ($\delta_B$), respectively. $\delta_{litter}$ is the $\delta^{13}C$ (or $\delta^{15}N$) of the initial litter. The amount of litter-derived C and N in these pools were obtained by multiplying the $f_{litter}$ values to corresponding C (or N) pool sizes.

For microbial biomass, $\delta^{13}C$ and $\delta^{15}N$ values of fumigated and nonfumigated subsamples contained were blank corrected using the following mixing model to calculate $\delta_A$ of the extract (DOM + microbial biomass):

$$\delta_A = \frac{\delta_S(C_A + C_B) - C_B\delta_B}{C_A} \tag{8}$$

where $\delta_S$ is the $\delta^{13}C$ or $\delta^{15}N$ of the extract, $C_A$ is the C or N concentration of the extract, $C_B$ is the C or N concentration of the blank, and $\delta_B$ is the $\delta^{13}C$ or $\delta^{15}N$ of the blank sample.

The calculation of $\delta^{13}C$ and $\delta^{15}N$ ($\delta_{mic}$) of the microbial biomass was done as described by Potthoff et al. (2003) using the following equation:

$$\delta_{mic} = \frac{\delta_f \times C_f - \delta_{nf} \times C_{nf}}{C_f - C_{nf}} \tag{9}$$

where $\delta_f$ and $\delta_{nf}$ are the $\delta^{13}C$ (or $\delta^{15}N$) of fumigated and nonfumigated extracts ($\delta_A$ from equation 9), and $C_f$ and $C_{nf}$ are the corresponding concentrations of C (or N), respectively (mg $g^{-1}$ soil). Calculation of litter-derived C and N in microbial biomass was done as described above.

### *2.11 Statistical analyses*

All statistical analyses, data manipulation, and data visualization were performed on R software version 4.4.0 (R Core Team, 2020), with the R packages "dplyr" (Wickham et al., 2019),"ggplot2" (Wickham et al., 2019), "lmerTest" (Kuznetsova et al., 2017), "performance" (Lüdecke et al., 2024),"lme4" (Bates et al., 2015), "rstatix" (Kassambara, 2023), and "emmeans" (Lenth et al., 2020).

To assess the impact of faunal treatments on both total and litter-derived respiration rates, as well as cumulative CO2 emissions, we employed repeated-measures ANOVA using linear mixed-effects models. Time and replicate were included as random effects to account for temporal variations and inherent differences among replicates. We assessed the effect of faunal treatments on litter-derived C and N contents of microbial biomass, amino sugars, and POM and MAOM using linear mixed effects models with Harvest as an interacting fixed effect and replicate as a random effect. Diagnostic plots of all models were produced using "performance" package to check assumptions of normality, homoscedasticity, and linearity. Variables were log-transformed as needed to meet the assumptions of ANOVA (homogeneity of variance and normality of residuals). Full and reduced mixed effects models were compared with Wald Chi-Square tests to approximate p-values. Pairwise least-square means comparison was conducted using Tukey's adjustment. For all statistical analysis, $p < 0.05$ was considered significant and $p < 0.1$ as marginally significant. The $\delta^{13}C$ value of $CO_2$ respiration was interpolated using "zoo" package in R for days $^{13}C$ was not measured (Zeileis et al., 2023).

## 3. RESULTS

### *3.1 Fauna manipulation treatments*

The thermal treatment employed to remove nematodes successfully eliminated all bacterial feeding nematodes in the 'no-fauna' treatment. However, we did recover fungal feeding and root feeding nematodes in low abundances in all treatments (Table 1). All nematodes were enriched in $^{13}C$ and $^{15}N$ after the 1 month and became more enriched by the end of the incubation, while their abundance decreased (Fig. 5.1; Table 5.1)

We found that mites did not have a significant effect on nematode abundance (Table 5.1; $t_{45} = -0.73$, $p = 0.745$), but did significantly increase the $\delta^{13}C$ and $\delta^{15}N$ enrichment of the bacterial feeding nematode population after 6 months of incubation (Fig. 5.1; $t_{29} = -4.36$, $p = 0.0001$).

**Table 5.1:** Nematode trophic group composition (individuals per 100g of dry soil) by harvest and treatment. Treatments include control with no fauna added ('no-fauna'), bacterial feeding nematode addition ('nem'), and nematode plus predatory mite addition ('mite'). Mean ± standard error presented, n = 6.

| Harvest | Treatment | Bacterial-feeders | Fungal-feeders | Root-feeders |
|---|---|---|---|---|
| 1 month | no-fauna | 0.0 | 67.0 ± 32.6 | 2.5 ± 0.7 |
| | nem | 5400.0 ± 517.6 | 50.2 ± 30.7 | 1.8 ± 1.2 |
| | mite | 5919.7 ± 805.4 | 16.3 ± 12.9 | 0 ± 0 |
| 6 month | no-fauna | 0.0 | 21.8 ± 9.5 | 0.7 ± 0.5 |
| | nem | 2210.8 ± 187.2 | 6.7 ± 2.8 | 0.0 |
| | mite | 2602.5 ± 224.0 | 9.2 ± 3.0 | 4.2 ± 2.0 |

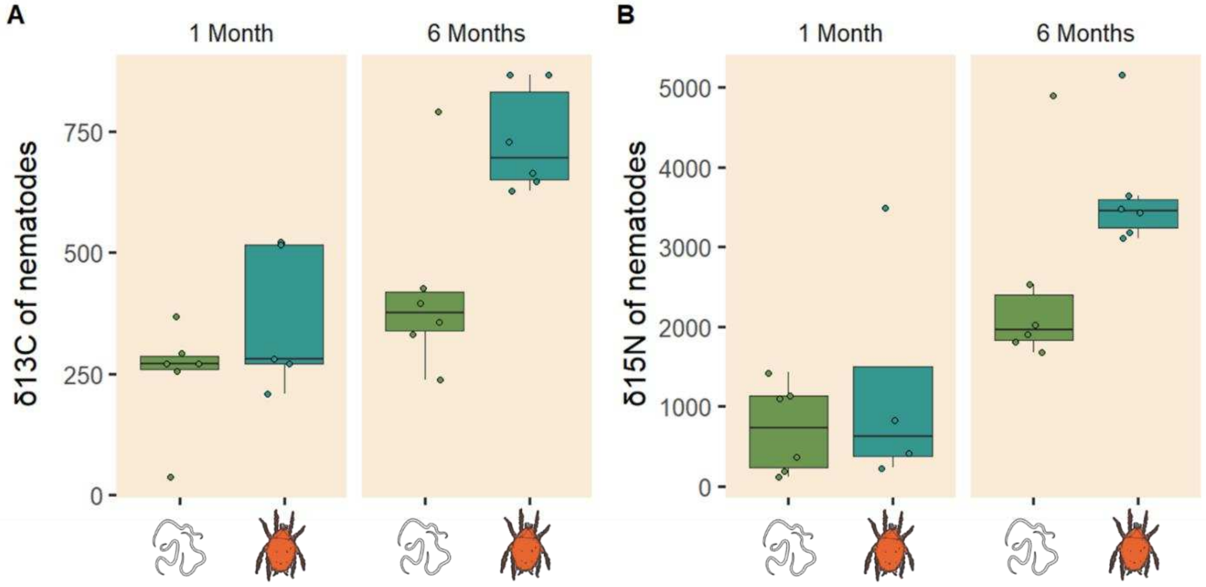


**Figure 5.1:** Stable isotope signatures of $\delta^{13}$C (**A**) and $\delta^{15}$N (**B**) of bacterial feeding nematodes measured at 1 month and 6 months under two different faunal treatments. Icons below the x-axis indicate the treatment type: nematodes for the nematode-only treatment ('nem') and a predatory mite for the combined nematode and mite treatment ('mite'). Box plots illustrate the median, upper and lower quartiles, and range.

### *3.2 Microbial activity*

To estimate the microbial activity, we quantified the effect of our treatments ('no-fauna', 'nem', and 'mite') on total respiration rate (µg $CO_2$-C g soil$^{-1}$ day$^{-1}$), and cumulative respiration (µg $CO_2$-C g soil$^{-1}$) throughout the duration of the incubation. Litter-derived respiration was ~53% of total respiration at the end of the 182 day incubation. We found that the treatments did not have significant effects on total respiration rates (Fig. 5.2A; $X^2 = 2.32$, $p = 0.314$), or litter-derived respiration rates (Fig. 5.2B; $X^2 = 2.56$, $p = 0.278$). However, we found that total cumulative respiration was highest in the 'nem' treatment (Fig. 5.3A; $X^2 = 191.2$), significantly higher than in the 'no-fauna' ($t_{52} = -10.48$, $p < 0.0001$) and 'mite' ($t_{52} = 13.05$, $p < 0.0001$) treatments. Interestingly, the 'no-fauna' treatment had the highest cumulative litter-derived respiration (Fig. 5.3B; $X^2 = 7.96$), significantly higher than 'nem' ($t_{52} = 2.32$, $p = 0.061$) and 'mite' ($t_{52} = 2.55$, $p = 0.036$). This difference in treatment effect between the total and litter-derived respiration resulted in a considerable increase in cumulative respiration derived from the native soil organic C pool under ‘nem’ (Fig. 5.3C; $X^2 = 239.8$), compared to ‘no-fauna’ ($t_{52} = -11.96$, $p < 0.0001$) and ‘mite’ ($t_{52} = 14.50$, $p < 0.0001$).

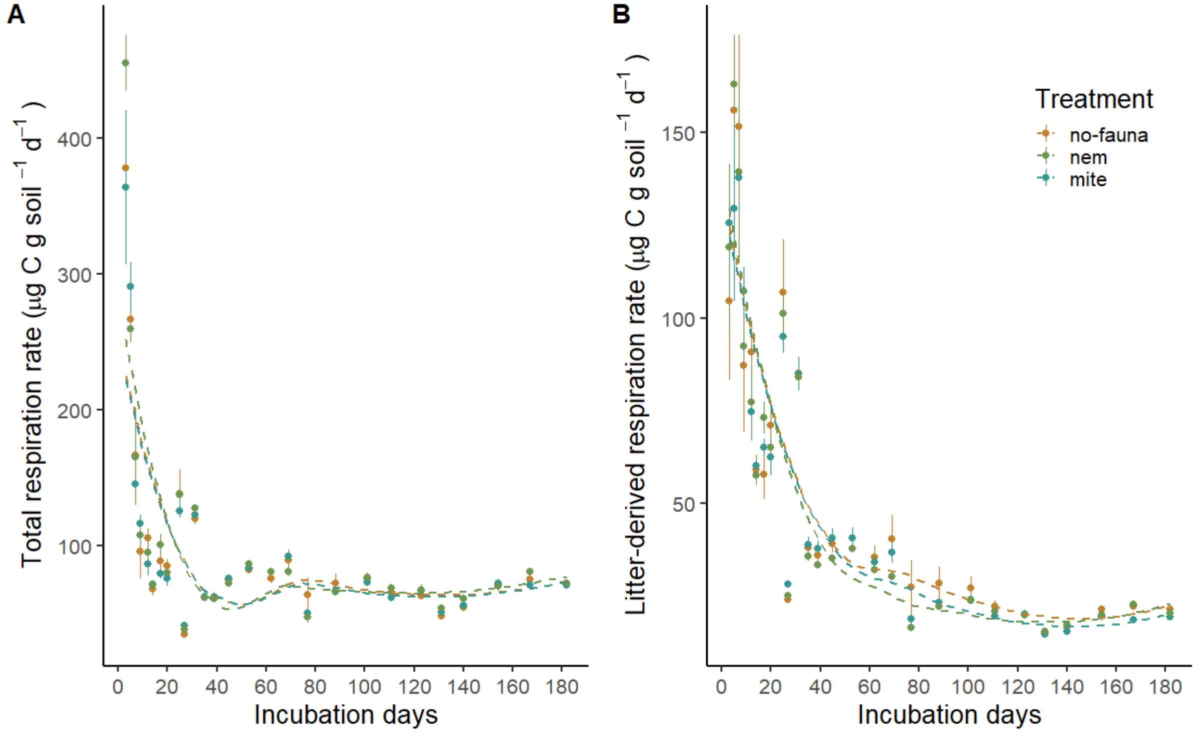


**Figure 5.2.** Effects of different soil fauna treatments on total soil respiration (**A**) and litter-derived respiration (**B**) rates (µg $CO_2$-C g soil$^{-1}$ day$^{-1}$) during the 182-day incubation period. Treatments include control with no fauna added (‘no-fauna’), bacterial feeding nematode addition (‘nem’), and nematode plus predatory mite addition (‘mite’), with dashed lines representing modeled decay curves showing the dynamics of respiration over time. Error bars depict the standard error among replicates (n = 6).

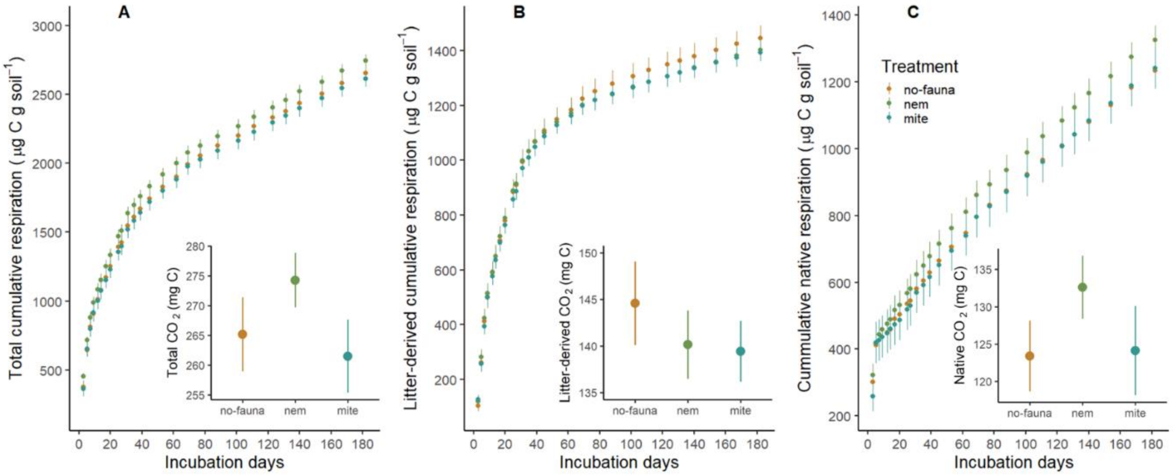


**Figure 5.3.** Effects of different soil fauna treatments on total cumulative respiration (**A**), litter-derived cumulative respiration (**B**), and native soil cumulative respiration (**C**) rates (µg CO2-C g soil$^{-1}$) during a 180-day incubation period. Treatments include control with no fauna added ('no-fauna'), bacterial feeding nematode addition ('nem'), and nematode plus predatory mite addition ('mite'). Each panel illustrates the cumulative $CO_2$ emissions across the incubation days, with insets showing the total CO2 released by the end of the period in each treatment in mg C. Error bars in main plots represent the standard error among replicates (n=6). Error bars in inserts represent 95% confidence interval around the mean.

### *3.3 Microbial biomass and necromass*

There was no significant difference in our proxies for total microbial biomass C ($X^2 = 0.44$, $p = 0.802$) or litter-derived microbial biomass C ($X^2 = 0.08$, $p = 0.963$) between the treatments (Fig. 5.4).

Bacterial necromass was greatest in the ‘mite’ treatment, which was significantly higher than the ‘nem’ treatment ($t_9 = -2.39$, $p = 0.087$), but not than the ‘no-fauna’ treatment ($t_9 = -1.96$, $p = 0.177$) (Fig. 5.5A). Bacterial necromass contributed 6.6% (CI: 6.2, 6.9%) to SOC in ‘mite’, 5.1% (CI: 4.1, 6.2%) in ‘no-fauna’, and 4.6% (CI: 2.7, 6.4%) in ‘nem’ and 14.8% (CI: 13.9, 15.6%), 11.3% (CI: 9.0, 13.6%), and 10.1% (CI: 6.2, 15.1%) to SON, respectively. Fungal necromass was greatest in ‘mite’ treatment also, but not significantly higher than the ‘no-fauna’ or ‘nem’ treatments (Fig. 5.5B; $F = 2.21$, $p = 0.166$). The contribution to SOM in the ‘mite’ treatment ($F = 5.12$, $p = 0.033$), was significantly higher than the ‘no-fauna’ ($t_9 = -2.82$, $p = 0.048$), and ‘nem’ ($t_9 = -2.72$, $p = 0.056$) treatments.

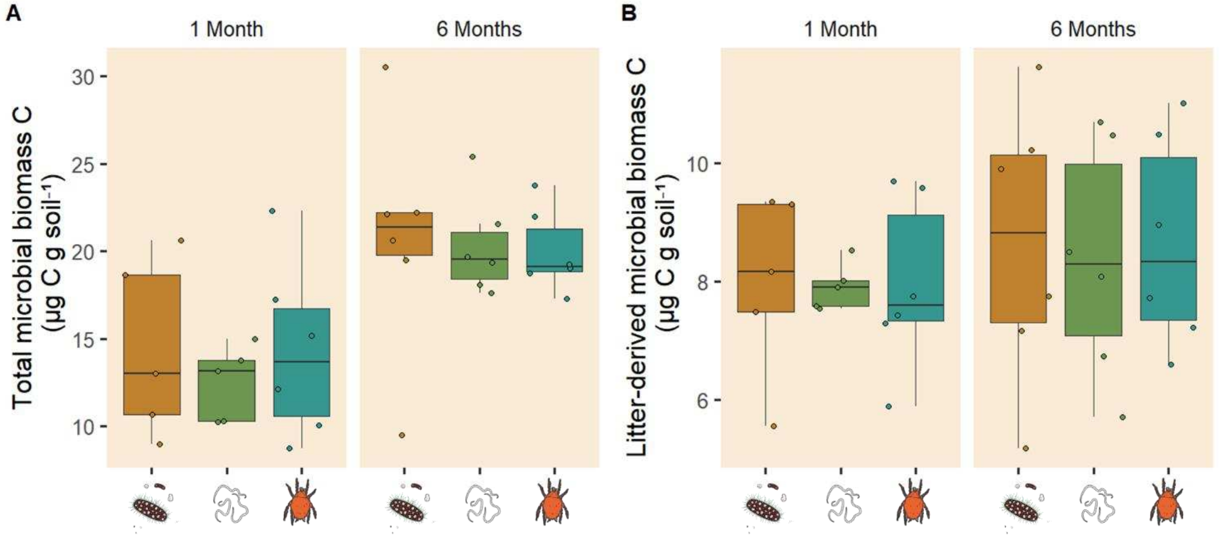


**Figure 5.4.** Effects of different soil fauna treatments on total microbial biomass (**A**) and litter-derived microbial biomass (**B**) carbon (µg C g soil$^{-1}$) , after 1 month and 6 months of incubation. Icons below the x-axis indicate the treatment type: bacteria for the 'no-fauna' treatment, nematodes for the nematode-only treatment ('nem') and a predatory mite for the combined nematode and mite treatment ('mite'). Box plots illustrate the median, upper and lower quartiles, and range.

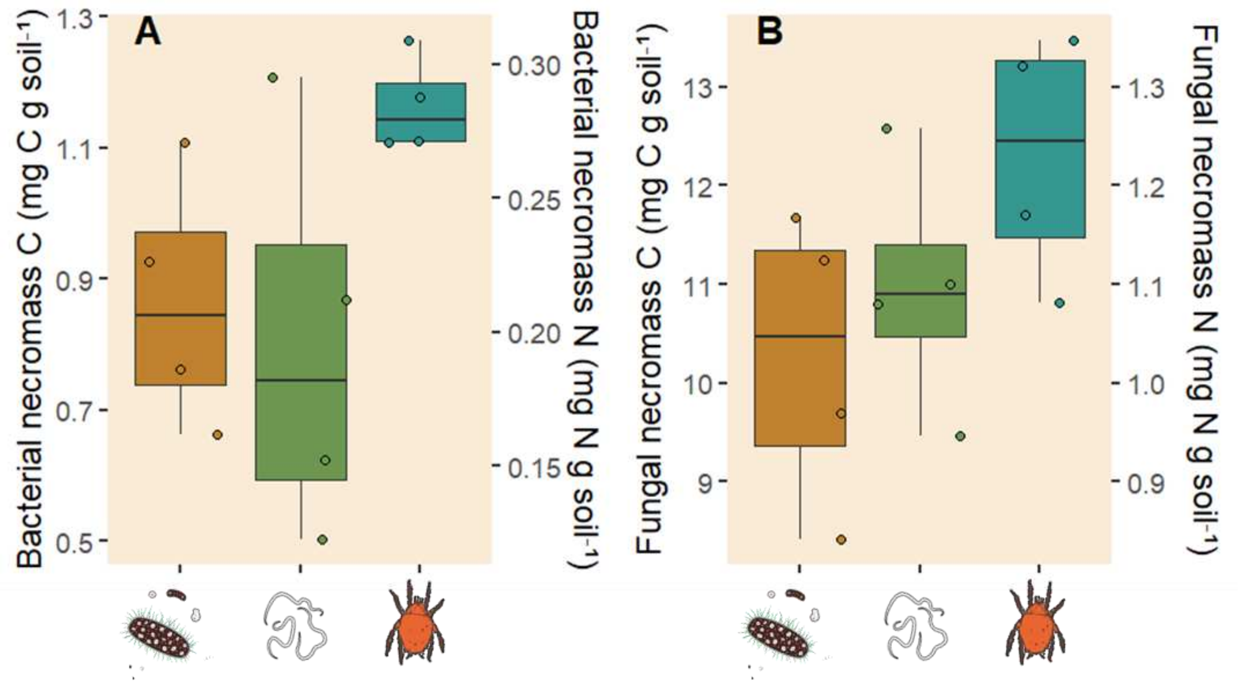


**Figure 5.5.** Effects of different soil fauna treatments on (A)bacterial necromass carbon and nitrogen (mg C|N g soil$^{-1}$) and (**B**) fungal necromass C and N (mg C|N g soil$^{-1}$) after 6 months of incubation. Icons below the x-axis indicate the treatment type: bacteria for the 'no-fauna' treatment, nematodes for the nematode-only treatment ('nem') and a predatory mite for the combined nematode and mite treatment ('mite'). Box plots illustrate the median, upper and lower quartiles, and range.

### *3.4 Effect on POM and MAOM*

Soil fauna treatments did not impact litter-derived bulk SOM ($F = 1.12$, $p = 0.341$) but had varied effects on both MAOM (Fig. 5.6; $F = 7.29$, $p = 0.003$) and POM (Fig. 5.7; $F = 3.88$, $p = 0.032$). After 1 month of incubation, both the 'mite' (mean = 0.524 mg C g soil$^{-1}$, $p = 0.001$) and 'nem' (mean = 0.504 mg C g soil$^{-1}$, $p = 0.017$) treatments had a significantly greater proportion of litter C in MAOM than 'no-fauna' (Fig. 5.6A; mean = 0.459 mg C g soil$^{-1}$). After 6 months of incubation, litter-derived MAOM C decreased (mean = 0.336 mg C g soil$^{-1}$) and there was no longer a difference between treatments (Fig. 5.6A; $F = 1.86$, $p = 0.191$). Conversely, no significant differences in litter-derived MAOM N were observed between treatments after 1 month (Fig. 5.6B; mean = 19.5 μg N g soil$^{-1}$, $p = 0.393$). However, litter-derived MAOM N increased over time and was highest in the 'mite' treatment after 6 months (Fig. 5.6B; mean = 27.7 μg N g soil$^{-1}$), significantly higher than 'no-fauna' (mean = 21.9 μg N g soil$^{-1}$, $p = 0.0007$), and 'nem' (mean = 23.4 μg N g soil$^{-1}$, $p = 0.0007$) treatments.

The proportion of litter C in POM after 1 month was greatest in the 'no-fauna' treatment (Fig. 5.7A; mean = 2.23 mg C g soil$^{-1}$), significantly higher than 'nem' (mean = 1.89 mg C g soil$^{-1}$, $p = 0.100$), but not 'mite' (mean = 2.01 mg C g soil$^{-1}$, $p = 0.353$). Similarly to litter derived MAOM C, the amount of litter C in POM decreased over time, and after six months the treatment effect was no longer significant (mean = 1.52 mg C g soil$^{-1}$, $F = 0.43$, $p = 0.61$). Litter derived POM N behaved like POM C rather than MAOM N; the proportion of litter-derived N in POM was highest in 'no-fauna' (Fig. 5.7B; mean = 42.8 μg N g soil$^{-1}$) after 1 month, significantly higher than 'nem' (mean = 35.3 μg N g soil$^{-1}$, $p = 0.005$), but not 'mite' (mean = 39.1 μg N g soil$^{-1}$, $p = 0.236$). Again, it decreased over time and after 6 months there was no difference between treatments (mean = 24.2 μg N g soil$^{-1}$, $F = 0.43$, $p = 0.61$).

After 1 month, the C:N of litter-derived MAOM was 25.9 in the ‘mite’ and 26.3 in the ‘nem’ treatments, slightly higher than the 24.5 C:N of the ‘no-fauna’ treatment, yet not significantly so ($F$ = 1.55, $p$ = 0.245). These new litter C:N values were similar to the 28.5 C:N of the added litter. However, after 6 months, the C:N of litter-derived MAOM shifted to 12.8 in ‘mite’, significantly lower than the 15.4 C:N of ‘no-fauna’ ($p$ = 0.042). The 13.9 C:N of ‘nem’ after 6 months was not significantly different from the other treatments ($p$ = 0.322). This shift in C:N of MAOM under ‘mite’ was evident in the 7.11 C:N of the total MAOM pool, marginally lower than the 7.39 C:N of ‘no-fauna’ ($p$ = 0.102). Moreover, the C:N of total POM pool was significantly higher under ‘mite’ (mean = 14.1, $p$ = 0.021) and ‘nem’ (mean = 14.0, $p$ = 0.039), as compared to ‘no-fauna’ (mean = 13.6) after 6 months of incubation.

We recovered approximately 89.0% of litter C and only 38.8% of litter N after 6 months of incubation (Fig. 5.8). In the 'no-fauna' treatment, MAOM comprised 9.9% C and 18.2% N, POM plus undecomposed litter accounted for 39.7% C and 18.7% N, and $CO_2$ respiration made up 39.0% C of the initial litter. In the 'nem’ treatment, MAOM comprised 9.4% C and 19.4% N, POM plus undecomposed litter accounted for 40.2% C and 17.9% N, and $CO_2$ respiration made up 37.8% C of the initial litter. In the ‘mite’ treatment, MAOM comprised 10.4% C and 22.8% N, POM plus undecomposed litter accounted for 43.0% C and 19.3% N, and $CO_2$ respiration made up 37.6% C of the initial litter.

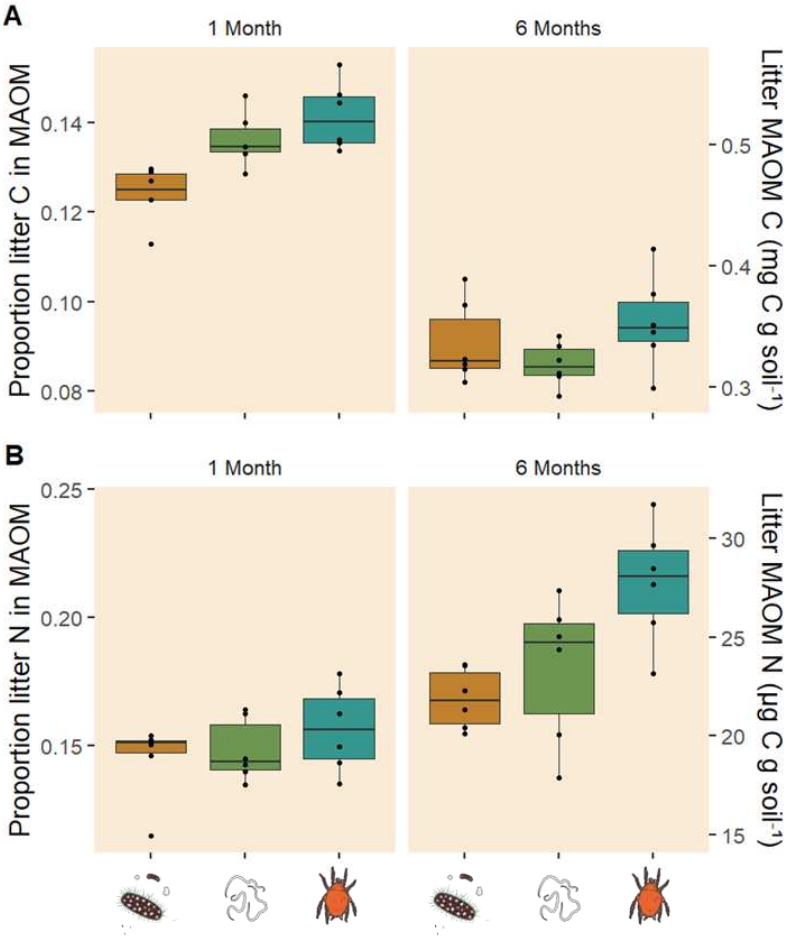


**Figure 5.6.** Effects of different soil fauna treatments on the proportion and amount (mg C | µg N g soil$^{-1}$) of litter-derived carbon (C) in mineral-associated organic matter (MAOM) (**A**) and litter-derived nitrogen (N) in MAOM (**B**), after 1 month and 6 months of incubation. Icons below the x-axis indicate the treatment type: bacteria for the 'no-fauna' treatment, nematodes for the nematode-only treatment ('nem') and a predatory mite for the combined nematode and mite treatment ('mite'). Box plots illustrate the median, upper and lower quartiles, and range.

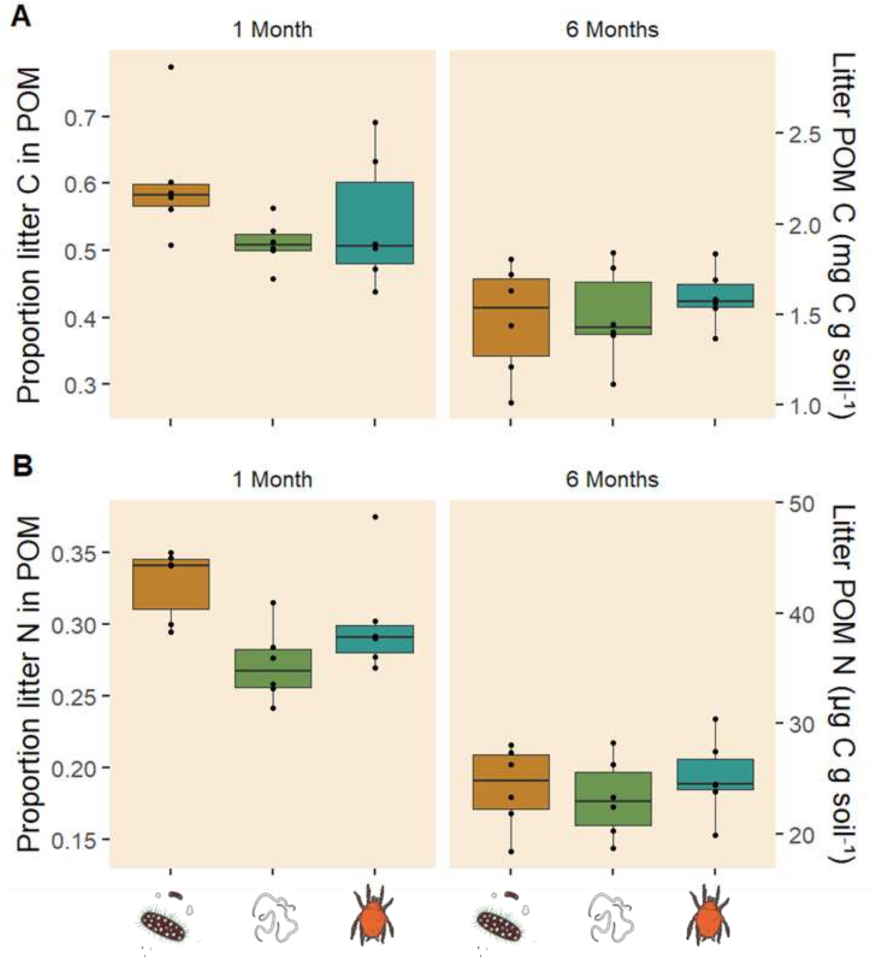


**Figure 5.7.** Effects of different soil fauna treatments on the proportion and amount (mg C | µg N g soil$^{-1}$) of litter-derived carbon (C) in particulate organic matter (POM) (**A**) and litter-derived nitrogen (N) in POM (**B**), after 1 month and 6 months of incubation. Icons below the x-axis indicate the treatment type: bacteria for the 'no-fauna' treatment, nematodes for the nematode-only treatment ('nem') and a predatory mite for the combined nematode and mite treatment ('mite'). Box plots illustrate the median, upper and lower quartiles, and range.

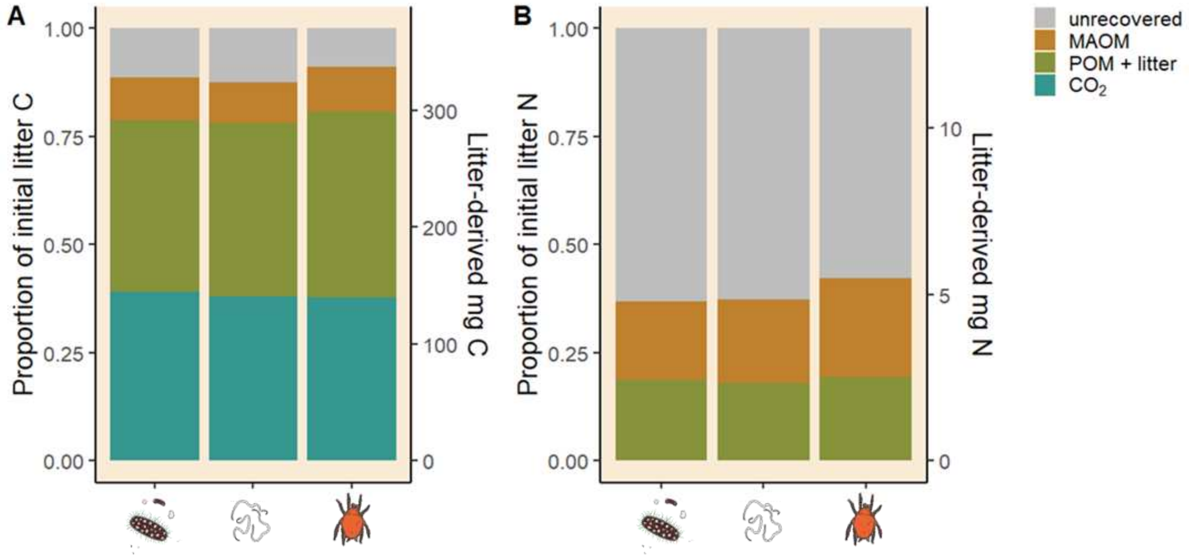


**Figure 5.8.** (**A**) Carbon and (**B**) nitrogen budgets after 6 months of incubation showing proportion of litter C and N among different pools: $CO_2$ respiration, particulate organic matter (POM), mineral-associated organic matter (MAOM), and unrecovered C or N. Icons below the x-axis indicate the treatment type: bacteria for the 'no-fauna' treatment, nematodes for the nematode-only treatment ('nem') and a predatory mite for the combined nematode and mite treatment ('mite').

## 4. DISCUSSION

In this study, we investigated the role of soil fauna in driving microbial necromass production and SOM persistence using stable isotopes to trace C and N flows through a simplified microcosm ecosystem. Our primary objectives were to determine how bacterivorous nematodes and predatory mites influence microbial activity, growth, and turnover, ultimately affecting SOM dynamics. Our findings revealed that bacterivorous nematodes stimulated microbial decomposition of native SOM, but not newly added litter (Fig. 5.2). Interestingly, the combination of nematodes and mites initially inhibited microbial uptake of litter C but later enhanced microbial turnover and necromass production (Fig. 5.5), leading to increased MAOM-N formation (Fig. 5.6). Notably, nematodes alone did not enhance microbial turnover and necromass formation; these effects were only observed in the presence of predatory mites. Our results provide insight into the intricate and dynamic interactions between soil fauna and microbial communities, emphasizing the likely importance of trophic interactions in SOM formation and stabilization.

### *4.1 Microbial activity responded to nematode pressure*

Our observations that nematodes significantly increase respiration rates of native SOC aligns with existing literature, which suggests that soil fauna can alter microbial processes through their trophic activities and interactions with microbial communities (Brussaard et al., 2007; Erktan et al., 2020). The significant increase in cumulative respiration of native SOC in the 'nem' treatment (Fig. 5.3A) suggests that nematodes stimulate microbes to accelerate the catabolism of SOM. This likely occurs because nematodes feed on bacteria, mineralizing N, which then becomes available to other microbes, supporting the synthesis of enzymes needed to degrade complex organic materials (Sinsabaugh, 2010). By this mechanism, nematode-bacteria interactions can significantly enhance the metabolic capacities of various C substrates, thereby C cycling (Jiang et al., 2018). Interestingly, nematodes did not stimulate the microbial respiration of native SOM when under the pressure of predatory mites. This indicates that mites either inhibit this effect of nematodes directly or modify nematode grazing behavior to indirectly alter the competitive dynamics and C use efficiency of the microbial community (Iven et al., 2022).

In contrast, the 'no-fauna' treatment showed the highest respiration rates for newly added organic matter (Fig. 5.2B), emphasizing the complex role soil fauna play in microbial C use efficiency (Sinsabaugh et al., 2016). The absence of fauna may reduce predation and competition pressures on microbes, potentially increasing their activity toward fresh and available C sources. Alternatively, the lack of available N from nematode mineralization means microbes must metabolize a significant amount of C to assimilate SOM at the correct C:N stoichiometry. This finding is consistent with other studies that observed that soil fauna could suppress microbial activity through direct consumption, affecting microbial access to fresh substrates (Crowther et al., 2012; Trap et al., 2016). This result emphasizes the role of soil fauna in modulating microbial resource allocation and enzymatic activity, possibly through changes in microbial community composition or metabolic prioritization (Hungate et al., 2021). Nematode-bacteria interactions have been shown to increase microbial C use efficiency through changes to microbial abundance and community composition by selective predation and providing new niches for colonization (Zheng et al., 2022; Shi et al., 2023).

This dual role of soil fauna, both facilitating and suppressing microbial activity, highlights the intricate balance soil fauna maintain in microbial C use efficiency. However, our study found that bacterivorous nematodes did not enhance microbial turnover and necromass formation unless predatory mites were present. The presence of mites resulted in peak microbial necromass formation, supporting our alternative hypothesis. This suggests that nematode-mite interactions play a crucial role in stimulating microbial turnover and enhancing MAOM formation through the management of nematode populations.

### *4.2 Predatory mites enhance nematode turnover*

Our study revealed no significant change in nematode abundance due to predatory mite exposure (Table 5.1), yet the observed $^{13}$C and $^{15}$N enrichment in nematodes (Fig. 5.1) suggests increased turnover and potential shifts in the community composition of bacterial-feeding nematodes. *Stratiolaelaps scimitus* are generalist predators and are likely targeting larger nematode individuals (Cabrera et al., 2005; Xie et al., 2018; Yang et al., 2020), potentially driving a shift in bacterivore community composition. This observation aligns with previous studies suggesting that predatory stress can lead to shifts in prey population structure, potentially favoring smaller, quicker-reproducing individuals (Laakso and Setälä, 1999), likely due to selective predation on larger nematodes (Hohberg and Traunspurger, 2005). In this study, it seems that predatory mites exert cascading effects on ecosystem processes, primarily by modifying and in some cases amplifying the impact of bacterivorous nematodes on the microbial community. This interaction suggests that predatory mites not only affect nematode behavior and survival but also indirectly influence microbial dynamics through altered nematode activity.

### *4.3 Nematodes under mite pressure drive microbial necromass accrual*

Although there was no shift in microbial biomass (Fig. 5.4), the enhanced necromass accrual under nematodes and mites indicates increased microbial turnover (Fig. 5.5). Interestingly, despite the impact of nematodes alone on microbial respiration, enhanced necromass formation was only evident under mite pressure. This suggests that mites are altering nematode grazing behavior and maintaining it in a state that stimulates microbial turnover, potentially through changes to nematode body size or reduction in grazing pressure (Laakso and Setälä, 1999; Hohberg and Traunspurger, 2005).

Bacterivorous nematodes with smaller body sizes are known to have a greater impact on mineralization rates and possibly lead to the release of organic N compounds (Brondani et al., 2022). Additionally, mites consuming nematodes must maintain homeostasis and mineralize additional N that would otherwise be trapped in the nematode biomass (Osler and Sommerkorn, 2007). This increased N availability likely stimulates microbial populations with faster growth rates and higher N contents. Their microbial products are thus more prone to binding to mineral surfaces upon cellular lysis from predation (Lambert, 2008; Daly et al., 2021). Additionally, there is some potential for mites to influence not only bacterial feeders but also fungal feeding nematodes which were present in small abundances in all treatments (Table 5.1), though this is not fully quantifiable due to insufficient data on fungal feeders. This broader impact of mite predation across different microbial communities highlights the need for further investigation (Wardle et al., 2004).

The interaction between predatory mites and nematodes shows the complexity of soil food webs in shaping microbial necromass accrual and SOM dynamics. The presence of predatory mites appears to amplify the effects of bacterivorous nematodes on microbial turnover, leading to enhanced necromass formation and potentially greater stabilization of organic matter in mineral-associated forms (Liang, 2020). This finding aligns with other research showing that higher trophic levels in the soil food web can have cascading effects on microbial anabolism and turnover rates, driving necromass formation (Kou et al., 2023). This study further demonstrates the synergistic effects of nematode-mite interactions, emphasizing the complexity and importance of multi-trophic interactions in soil ecosystems (Lucas et al., 2020).

### *4.4 Soil fauna enhance MAOM formation and alter SOM stoichiometry*

Our microcosm experiment revealed that the presence of bacterivorous nematodes and predatory mites significantly stimulated microbial activity and turnover, leading to enhanced production of microbial necromass. This aligns with previous studies indicating that microbial grazing by soil fauna can lead to increased microbial turnover and potentially increase the input of microbial-derived compounds to the soil organic matter pool (Djigal et al., 2004; Trap et al., 2016). Specifically, the introduction of nematodes and mites increased MAOM formation (Fig. 5.6B), suggesting that MAOM may accrue more quickly from materials that are highly processed by microbes.

Initially, the presence of mites alongside nematodes led to greater MAOM-C content, possibly by suppressing microbial uptake of litter dissolved organic matter (DOM) leached early during decomposition (Cotrufo et al., 2015). The C:N ratio of the new MAOM (Fig. 5.6), which was similar to that of the new litter, suggests direct sorption of plant DOM to minerals, bypassing the microbial pool. This is supported by the higher respiration of litter-derived $CO_2$ in the 'no-fauna' treatment. Further, the greater proportion of litter C remaining in POM in the 'no-fauna' treatment after one month (Fig. 5.7A), also points to a suppressed microbial activity in the 'mite' and 'nem' treatments.

Later in decomposition as the MAOM C:N decreased, this plant-derived MAOM was likely exchanged for compounds with higher N content, suggesting that microbially-derived MAOM may form later and has greater stability. After six months, POM levels were consistent across treatments (Fig. 5.7), but MAOM-N increased (Fig. 5.6B) and C:N stoichiometry was lower with nematodes and mites. This indicates that soil fauna, through their effects on microbes, can efficiently convert POM-N into microbial products that form MAOM-N. MAOM is increasingly recognized as both a storage reserve and a vital source of bioavailable N (Jilling et al., 2018, 2021). The mechanisms of N entry and exit from the MAOM pool require further research, but likely involve complex interactions between soil health management, mineral surfaces, microbes, plant roots, and soil fauna (Grandy et al., 2022). This MAOM-

N entry driven by soil fauna in our study highlights the crucial role of trophic interactions in soil nutrient cycling and MAOM N, which could be critical for long-term soil fertility.

Our study showed no effect on total SOM, but there were measurable impacts on MAOM and POM. These minor shifts in formation and stabilization can have long-term effects at scale (Cotrufo et al., 2019; Lugato et al., 2021; Angst et al., 2023; Hansen et al., 2024; Li et al., 2024). Soil C recovery was good, with some unrecovered C likely due to fraction recovery and $CO_2$ leakage (Fig. 5.8A). However, N recovery was poor, indicating another flux of N out of the system (Fig. 5.8B). Some N was likely lost as DOM during fractionation, but volatilization of ammonia or off gassing of N through denitrification processes could also account for the losses. Grazing pressure by nematodes has been shown to reduce the relative abundance of denitrifying bacteria, as well as reduce denitrification enzyme activity (Djigal et al., 2010). It is possible that the higher N recovery in the mite treatment (Fig. 5.8B) is the result of decreased denitrification; however, without measurements of $NH_3$, $N_2$, or $N_2O$, the exact pathways remain uncertain.

In summary, our hypothesis that bacterivorous nematodes alone would decrease microbial biomass, enhance activity, and drive turnover and necromass formation leading to greater MAOM formation was not fully supported. However, our alternative hypothesis that the addition of mite predators would result in peak microbial turnover and necromass formation and enhance MAOM formation was supported. These findings reveal the critical role of soil fauna in enhancing N stabilization within MAOM, reflecting their broader ecological impacts on SOM transformation and stabilization processes (Kögel-Knabner et al., 2008). While bacterivores initially enhanced MAOM-C formation, this effect diminished over time, suggesting a transient or equilibrium effect on C stabilization. In contrast, the sustained higher levels of MAOM-N in the presence of mites suggest a prolonged influence on N stabilization, potentially through altered microbial decomposition pathways or enhanced microbial efficiency in N use.

### *4.5 Future research directions*

Despite the advances made in this study, several questions remain. The varying effects of bacterivores and predatory mites over different time scales suggest that longer-term studies are needed to fully understand the ecological and biogeochemical implications of faunal presence in soil systems. Expanding the range of soil types and climatic conditions in future studies could help generalize these findings and better predict the global implications of faunal interactions on soil C and N dynamics.

Future research should focus on elucidating the specific mechanisms by which nematodes and other soil fauna influence microbial enzymatic activities and SOM decomposition pathways. Experimental studies using isotopic tracers could provide more detailed insights into how N mineralization by soil fauna affects microbial degradation of different SOC components. Additionally, integrating comprehensive microbial community analyses, such as metagenomics and metabolomics, could delineate the pathways through which soil fauna influence microbial degradation of organic matter. Incorporating varying levels of faunal and microbial diversity in experimental designs could clarify the relative contributions of these biotic components to SOC dynamics. Studies incorporating stable isotope probing could elucidate the specific compounds degraded by microbes under the influence of soil fauna, providing a clearer picture of the biochemical pathways involved (Eisenhauer et al., 2017). Understanding these mechanisms is crucial for incorporating the role of fauna into biogeochemical models to better predict SOM dynamics (Grandy et al., 2016). Our study provides evidence and a replicable experimental design for examining the effects of soil fauna on microbial activity and turnover, advancing our understanding of SOM stabilization.

## 5. CONCLUSIONS

Overall, our study highlights the complex interplay between predatory mites and bacterivorous nematodes in shaping microbial necromass accrual and SOM dynamics. These interactions underscore the importance of considering multiple trophic levels in soil ecological studies to fully understand the mechanisms driving SOM formation and stabilization. Nematodes inhibit microbial uptake of labile DOM early in decomposition, leading to its incorporation into MAOM, especially under mite influence. They also primed the decomposition of native SOM by increasing nitrogen availability, stimulating microbial turnover and necromass formation. This results in increased MAOM-N and altered C:N stoichiometry indicating a more microbially derived MAOM. Our findings emphasize the critical role of the soil food web in mediating C and N dynamics, essential for predicting and managing soil responses to environmental changes. This nuanced understanding provides valuable insights into ecosystem functioning and the potential for enhancing MAOM-N in managed systems. Rather than focusing solely on microbes, biological interventions for soil health and SOM accrual could consider the entire food web. Ultimately, our study adds to the growing body of knowledge on soil food web interactions and their pivotal roles in regulating SOM dynamics.

## REFERENCES

Angst, G., Angst, Š., Frouz, J., Jabinski, S., Jílková, V., Kukla, J., Li, M., Meador, T.B., Angel, R., 2024. Stabilized microbial necromass in soil is more strongly coupled with microbial diversity than the bioavailability of plant inputs. Soil Biology and Biochemistry 190, 109323. doi:10.1016/j.soilbio.2024.109323

Angst, G., Mueller, K.E., Castellano, M.J., Vogel, C., Wiesmeier, M., Mueller, C.W., 2023. Unlocking complex soil systems as carbon sinks: multi-pool management as the key. Nature Communications 14, 2967. doi:10.1038/s41467-023-38700-5

Angst, Š., Mueller, C.W., Cajthaml, T., Angst, G., Lhotáková, Z., Bartuška, M., Špaldoňová, A., Frouz, J., 2017. Stabilization of soil organic matter by earthworms is connected with physical protection rather than with chemical changes of organic matter. Geoderma 289, 29–35. doi:10.1016/j.geoderma.2016.11.017

Azevedo, L.H., Moreira, M.F.P., Pereira, G.G., Borges, V., de Moraes, G.J., Inomoto, M.M., Vicente, M.H., de Siqueira Pinto, M., Peres, L.E.P., Rueda-Ramírez, D., Carta, L., Meyer, S.L.F., Mowery, J., Bauchan, G., Ochoa, R., Palevsky, E., 2020. Combined releases of soil predatory mites and provisioning of free-living nematodes for the biological control of root-knot nematodes on 'Micro Tom tomato.' Biological Control 146, 104280. doi:10.1016/j.biocontrol.2020.104280

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. doi:10.18637/jss.v067.i01

Brondani, M., Plassard, C., Ramstein, E., Cousson, A., Hedde, M., Bernard, L., Trap, J., 2022. Morpho-anatomical traits explain the effects of bacterial-feeding nematodes on soil bacterial community composition and plant growth and nutrition. Geoderma 425, 116068. doi:10.1016/j.geoderma.2022.116068

Brussaard, L., Pulleman, M.M., Ouédraogo, É., Mando, A., Six, J., 2007. Soil fauna and soil function in the fabric of the food web. Pedobiologia 50, 447–462. doi:10.1016/j.pedobi.2006.10.007

Buckeridge, K.M., Mason, K.E., McNamara, N.P., Ostle, N., Puissant, J., Goodall, T., Griffiths, R.I., Stott, A.W., Whitaker, J., 2020. Environmental and microbial controls on microbial necromass recycling, an important precursor for soil carbon stabilization. Communications Earth & Environment 1, 1–9. doi:10.1038/s43247-020-00031-4

Cabrera, A.R., Cloyd, R.A., Zaborski, E.R., 2005. Development and reproduction of Stratiolaelaps scimitus (Acari: Laelapidae) with fungus gnat larvae (Diptera: Sciaridae), potworms (Oligochaeta: Enchytraeidae) or Sancassania aff. sphaerogaster (Acari: Acaridae) as the sole food source. Experimental & Applied Acarology 36, 71–81. doi:10.1007/s10493-005-0242-x

Camenzind, T., Mason-Jones, K., Mansour, I., Rillig, M.C., Lehmann, J., 2023. Formation of necromass-derived soil organic carbon determined by microbial death pathways. Nature Geoscience 16, 115–122. doi:10.1038/s41561-022-01100-3

Coleman, D.C., Crossley, D.A., Hendrix, P.F., 2004. 4 - Secondary Production: Activities of Heterotrophic Organisms—The Soil Fauna, in: Coleman, D.C., Crossley, D.A., Hendrix, P.F. (Eds.), Fundamentals of Soil Ecology (Second Edition). Academic Press, Burlington, pp. 79–185. doi:10.1016/B978-012179726-3/50005-8

Cotrufo, M.F., Ranalli, M.G., Haddix, M.L., Six, J., Lugato, E., 2019. Soil carbon storage informed by particulate and mineral-associated organic matter. Nature Geoscience 12, 989–994. doi:10.1038/s41561-019-0484-6

Cotrufo, M.F., Soong, J.L., Horton, A.J., Campbell, E.E., Haddix, M.L., Wall, D.H., Parton, W.J., 2015. Formation of soil organic matter via biochemical and physical pathways of litter mass loss. Nature Geoscience 8, 776–779. doi:10.1038/ngeo2520

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988–995. doi:10.1111/gcb.12113

Crossley, D.A., Blair, J.M., 1991. A high-efficiency, “low-technology” Tullgren-type extractor for soil microarthropods. Agriculture, Ecosystems & Environment, Proceedings of the International Workshop on Modern Techniques in Soil Ecology Relevant to Organic Matter Breakdown, Nutrient Cycling and Soil Biological Processes 34, 187–192. doi:10.1016/0167-8809(91)90104-6

Crowther, T.W., Boddy, L., Hefin Jones, T., 2012. Functional and ecological consequences of saprotrophic fungus–grazer interactions. The ISME Journal 6, 1992–2001. doi:10.1038/ismej.2012.53

Crowther, T.W., Thomas, S.M., Maynard, D.S., Baldrian, P., Covey, K., Frey, S.D., van Diepen, L.T.A., Bradford, M.A., 2015. Biotic interactions mediate soil microbial feedbacks to climate change. Proceedings of the National Academy of Sciences 112, 7033–7038. doi:10.1073/pnas.1502956112

Daly, A.B., Jilling, A., Bowles, T.M., Buchkowski, R.W., Frey, S.D., Kallenbach, C.M., Keiluweit, M., Mooshammer, M., Schimel, J.P., Grandy, A.S., 2021. A holistic framework integrating plant-microbe-mineral regulation of soil bioavailable nitrogen. Biogeochemistry 154, 211–229. doi:10.1007/s10533-021-00793-9

Deng, F., Liang, C., 2022. Revisiting the quantitative contribution of microbial necromass to soil carbon pool: Stoichiometric control by microbes and soil. Soil Biology and Biochemistry 108486. doi:10.1016/j.soilbio.2021.108486

Djigal, D., Baudoin, E., Philippot, L., Brauman, A., Villenave, C., 2010. Shifts in size, genetic structure and activity of the soil denitrifier community by nematode grazing. European Journal of Soil Biology 46, 112–118. doi:10.1016/j.ejsobi.2009.12.001

Djigal, D., Brauman, A., Diop, T.A., Chotte, J.L., Villenave, C., 2004. Influence of bacterial-feeding nematodes (Cephalobidae) on soil microbial communities during maize growth. Soil Biology and Biochemistry 36, 323–331. doi:10.1016/j.soilbio.2003.10.007

Eisenhauer, N., Antunes, P.M., Bennett, A.E., Birkhofer, K., Bissett, A., Bowker, M.A., Caruso, T., Chen, B., Coleman, D.C., de Boer, W., de Ruiter, P., DeLuca, T.H., Frati, F., Griffiths, B.S., Hart, M.M., Hättenschwiler, S., Haimi, J., Heethoff, M., Kaneko, N., Kelly, L.C., Leinaas, H.P., Lindo, Z., Macdonald, C., Rillig, M.C., Ruess, L., Scheu, S., Schmidt, O., Seastedt, T.R., van Straalen, N.M., Tiunov, A.V., Zimmer, M., Powell, J.R., 2017. Priorities for research in soil ecology. Pedobiologia 63, 1–7.

Erktan, A., Rillig, M.C., Carminati, A., Jousset, A., Scheu, S., 2020. Protists and collembolans alter microbial community composition, C dynamics and soil aggregation in simplified consumer–prey systems. Biogeosciences 17, 4961–4980. doi:https://doi.org/10.5194/bg-17-4961-2020

Ferris, H., Venette, R.C., van der Meulen, H.R., Lau, S.S., 1998. Nitrogen mineralization by bacterial-feeding nematodes: verification and measurement. Plant and Soil 203, 159–171. doi:10.1023/A:1004318318307

Franco, A.L.C., Knox, M.A., Andriuzzi, W.S., de Tomasel, C.M., Sala, O.E., Wall, D.H., 2017. Nematode exclusion and recovery in experimental soil microcosms. Soil Biology and Biochemistry 108, 78–83. doi:10.1016/j.soilbio.2017.02.001

Frouz, J., 2018. Effects of soil macro- and mesofauna on litter decomposition and soil organic matter stabilization. Geoderma 332, 161–172. doi:10.1016/j.geoderma.2017.08.039

Fulton-Smith, S., Cotrufo, M.F., 2019. Pathways of soil organic matter formation from above and belowground inputs in a Sorghum bicolor bioenergy crop. GCB Bioenergy 11, 971–987. doi:10.1111/gcbb.12598

Grandy, A.S., Daly, A.B., Bowles, T.M., Gaudin, A.C.M., Jilling, A., Leptin, A., McDaniel, M.D., Wade, J., Waterhouse, H., 2022. The nitrogen gap in soil health concepts and fertility measurements. Soil Biology and Biochemistry 175, 108856. doi:10.1016/j.soilbio.2022.108856

Grandy, A.S., Wieder, W.R., Wickings, K., Kyker-Snowman, E., 2016. Beyond microbes: Are fauna the next frontier in soil biogeochemical models? Soil Biology and Biochemistry, Special issue: Food web interactions in the root zone: influences on community and ecosystem dynamics 102, 40–44. doi:10.1016/j.soilbio.2016.08.008

Haddix, M.L., Gregorich, E.G., Helgason, B.L., Janzen, H., Ellert, B.H., Francesca Cotrufo, M., 2020. Climate, carbon content, and soil texture control the independent formation and persistence of particulate and mineral-associated organic matter in soil. Geoderma 363, 114–160. doi:10.1016/j.geoderma.2019.114160

Hansen, P.M., Even, R., King, A.E., Lavallee, J., Schipanski, M., Cotrufo, M.F., 2024. Distinct, direct and climate-mediated environmental controls on global particulate and mineral-associated organic carbon storage. Global Change Biology 30, e17080. doi:10.1111/gcb.17080

Hohberg, K., Traunspurger, W., 2005. Predator–prey interaction in soil food web: functional response, size-dependent foraging efficiency, and the influence of soil texture. Biology and Fertility of Soils 41, 419–427. doi:10.1007/s00374-005-0852-9

Hungate, B.A., Marks, J.C., Power, M.E., Schwartz, E., van Groenigen, K.J., Blazewicz, S.J., Chuckran, P., Dijkstra, P., Finley, B.K., Firestone, M.K., Foley, M., Greenlon, A., Hayer, M., Hofmockel, K.S., Koch, B.J., Mack, M.C., Mau, R.L., Miller, S.N., Morrissey, E.M., Propster, J.R., Purcell, A.M., Sieradzki, E., Starr, E.P., Stone, B.W.G., Terrer, C., Pett-Ridge, J., 2021. The Functional Significance of Bacterial Predators. MBio 12, e00466-21. doi:10.1128/mBio.00466-21

Iven, H., Walker, T.W.N., Anthony, M., 2022. Biotic interactions in soil are underestimated drivers of microbial carbon use efficiency. Current Microbiology 80, 13. doi:10.1007/s00284-022-02979-2

Jiang, Y., Liu, M., Zhang, J., Chen, Y., Chen, X., Chen, L., Li, H., Zhang, X.-X., Sun, B., 2017. Nematode grazing promotes bacterial community dynamics in soil at the aggregate level. The ISME Journal 11, 2705–2717. doi:10.1038/ismej.2017.120

Jiang, Y., Zhou, H., Chen, L., Yuan, Y., Fang, H., Luan, L., Chen, Y., Wang, X., Liu, M., Li, H., Peng, X., Sun, B., 2018. Nematodes and microorganisms interactively stimulate soil organic carbon turnover in the macroaggregates. Frontiers in Microbiology 9, 2803. doi:10.3389/fmicb.2018.02803

Jilling, A., Keiluweit, M., Contosta, A.R., Frey, S., Schimel, J., Schnecker, J., Smith, R.G., Tiemann, L., Grandy, A.S., 2018. Minerals in the rhizosphere: overlooked mediators of soil nitrogen availability to plants and microbes. Biogeochemistry 139, 103–122. doi:10.1007/s10533-018-0459-5

Jilling, A., Keiluweit, M., Gutknecht, J.L.M., Grandy, A.S., 2021. Priming mechanisms providing plants and microbes access to mineral-associated organic matter. Soil Biology and Biochemistry 158, 108265. doi:10.1016/j.soilbio.2021.108265

Kassambara, A., 2023. rstatix: Pipe-Friendly Framework for Basic Statistical Tests.

Kästner, M., Miltner, A., 2018. Chapter 5 - SOM and Microbes—What Is Left From Microbial Life, in: Garcia, C., Nannipieri, P., Hernandez, T. (Eds.), The Future of Soil Carbon. Academic Press, pp. 125–163. doi:10.1016/B978-0-12-811687-6.00005-5

Kästner, M., Miltner, A., Thiele-Bruhn, S., Liang, C., 2021. Microbial necromass in soils—Linking microbes to soil processes and carbon turnover. Frontiers in Environmental Science 9. doi:10.3389/fenvs.2021.756378

Kögel-Knabner, I., Guggenberger, G., Kleber, M., Kandeler, E., Kalbitz, K., Scheu, S., Eusterhues, K., Leinweber, P., 2008. Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. Journal of Plant Nutrition and Soil Science 171, 61–82. doi:10.1002/jpln.200700048

Kou, X., Morriën, E., Tian, Y., Zhang, X., Lu, C., Xie, H., Liang, W., Li, Q., Liang, C., 2023. Exogenous carbon turnover within the soil food web strengthens soil carbon sequestration through microbial necromass accumulation. Global Change Biology 29, 4069–4080. doi:10.1111/gcb.16749

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest Package: Tests in linear mixed effects models. Journal of Statistical Software 82, 1–26. doi:10.18637/jss.v082.i13

Laakso, J., Setälä, H., 1999. Population- and ecosystem-level effects of predation on microbial-feeding nematodes. Oecologia 120, 279–286. doi:10.1007/s004420050859

Lambert, J.-F., 2008. Adsorption and Polymerization of Amino Acids on Mineral Surfaces: A Review. Origins of Life and Evolution of Biospheres 38, 211–242. doi:10.1007/s11084-008-9128-3

Lavallee, J.M., Soong, J.L., Cotrufo, M.F., 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. Global Change Biology 26, 261–273. doi:10.1111/gcb.14859

Lehman, R.M., Cambardella, C.A., Stott, D.E., Acosta-Martinez, V., Manter, D.K., Buyer, J.S., Maul, J.E., Smith, J.L., Collins, H.P., Halvorson, J.J., Kremer, R.J., Lundgren, J.G., Ducey, T.F., Jin, V.L., Karlen, D.L., 2015. Understanding and enhancing soil biological health: The solution for reversing soil degradation. Sustainability 7, 988–1027. doi:10.3390/su7010988

Lenth, R., Buerkner, P., Herve, M., Love, J., Riebl, H., Singmann, H., 2020. emmeans: Estimated Marginal Means, aka Least-Squares Means.

Leuthold, S., Lavallee, J.M., Haddix, M.L., Cotrufo, M.F., 2024. Contrasting properties of soil organic matter fractions isolated by different physical separation methodologies. Geoderma 445, 116870. doi:10.1016/j.geoderma.2024.116870

Leuthold, S.J., Haddix, M.L., Lavallee, J., Cotrufo, M.F., 2022. Physical fractionation techniques, in: Reference Module in Earth Systems and Environmental Sciences. Elsevier. doi:10.1016/B978-0-12-822974-3.00067-7

Li, X., Gao, Y., Liu, Z., Liu, J., 2024. Enhanced soil carbon stability through alterations in components of particulate and mineral-associated organic matter in reclaimed saline–alkali drainage ditches. Agronomy 14, 869. doi:10.3390/agronomy14040869

Liang, C., 2020. Soil microbial carbon pump: Mechanism and appraisal. Soil Ecology Letters 2, 241–254. doi:10.1007/s42832-020-0052-4

Liang, C., Amelung, W., Lehmann, J., Kästner, M., 2019. Quantitative assessment of microbial necromass contribution to soil organic matter. Global Change Biology 25, 3578–3590. doi:10.1111/gcb.14781

Liang, C., Balser, T.C., 2011. Microbial production of recalcitrant organic matter in global soils: implications for productivity and climate policy. Nature Reviews Microbiology 9, 75–75. doi:10.1038/nrmicro2386-c1

Liang, C., Kästner, M., Joergensen, R.G., 2020. Microbial necromass on the rise: The growing focus on its role in soil organic matter development. Soil Biology and Biochemistry 150, 108000. doi:10.1016/j.soilbio.2020.108000

Liang, C., Read, H.W., Balser, T.C., 2012. GC-based detection of aldononitrile acetate derivatized glucosamine and muramic acid for microbial residue determination in soil. Journal of Visualized Experiments : JoVE. doi:10.3791/3767

Lucas, J.M., McBride, S.G., Strickland, M.S., 2020. Trophic level mediates soil microbial community composition and function. Soil Biology and Biochemistry 143, 107756. doi:10.1016/j.soilbio.2020.107756

Lüdecke, D., Makowski, D., Ben-Shachar, M.S., Patil, I., Waggoner, P., Wiernik, B.M., Thériault, R., Arel-Bundock, V., Jullum, M., Bacher, E., 2024. performance: Assessment of regression models performance.

Lugato, E., Lavallee, J.M., Haddix, M.L., Panagos, P., Cotrufo, M.F., 2021. Different climate sensitivity of particulate and mineral-associated soil organic matter. Nature Geoscience 14, 295–300. doi:10.1038/s41561-021-00744-x

Miltner, A., Bombach, P., Schmidt-Brücken, B., Kästner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111, 41–55. doi:10.1007/s10533-011-9658-z

Osler, G.H.R., Sommerkorn, M., 2007. Toward a complete soil C and N cycle: incorporating the soil fauna. Ecology 88, 1611–1621. doi:10.1890/06-1357.1

Peng, X., Gao, S., Ma, S., Liao, H., Liu, Y.-R., Chen, W., Huang, Q., Hao, X., 2024. Repeated labile carbon inputs trigger soil microbial necromass decomposition through increasing microbial diversity and hierarchical interactions. Soil Biology and Biochemistry 191, 109344. doi:10.1016/j.soilbio.2024.109344

Poeplau, C., Don, A., Six, J., Kaiser, M., Benbi, D., Chenu, C., Cotrufo, M.F., Derrien, D., Gioacchini, P., Grand, S., Gregorich, E., Griepentrog, M., Gunina, A., Haddix, M., Kuzyakov, Y., Kühnel, A., Macdonald, L.M., Soong, J., Trigalet, S., Vermeire, M.-L., Rovira, P., van Wesemael, B., Wiesmeier, M., Yeasmin, S., Yevdokimov, I., Nieder, R., 2018. Isolating organic carbon fractions with varying turnover rates in temperate agricultural soils – A comprehensive method comparison. Soil Biology and Biochemistry 125, 10–26. doi:10.1016/j.soilbio.2018.06.025

Potthoff, M., Loftfield, N., Buegger, F., Wick, B., John, B., Joergensen, R.G., Flessa, H., 2003. The determination of δ13C in soil microbial biomass using fumigation-extraction. Soil Biology and Biochemistry 35, 947–954. doi:10.1016/S0038-0717(03)00151-2

Sackett, T.E., Classen, A.T., Sanders, N.J., 2010. Linking soil food web structure to above- and belowground ecosystem processes: a meta-analysis. Oikos 119, 1984–1992. doi:10.1111/j.1600-0706.2010.18728.x

Shi, G., Luan, L., Zhu, G., Zeng, Z., Zheng, J., Shi, Y., Sun, B., Jiang, Y., 2023. Interaction between nematodes and bacteria enhances soil carbon sequestration under organic material amendments. Frontiers in Microbiology 14. doi:10.3389/fmicb.2023.1155088

Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42, 391–404. doi:10.1016/j.soilbio.2009.10.014

Sinsabaugh, R.L., Manzoni, S., Moorhead, D.L., Richter, A., 2013. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. Ecology Letters 16, 930–939. doi:https://doi.org/10.1111/ele.12113

Sinsabaugh, R.L., Turner, B.L., Talbot, J.M., Waring, B.G., Powers, J.S., Kuske, C.R., Moorhead, D.L., Follstad Shah, J.J., 2016. Stoichiometry of microbial carbon use efficiency in soils. Ecological Monographs 86, 172–189. doi:10.1890/15-2110.1

Soong, J.L., Nielsen, U.N., 2016. The role of microarthropods in emerging models of soil organic matter. Soil Biology and Biochemistry, Special issue: Food web interactions in the root zone: influences on community and ecosystem dynamics 102, 37–39. doi:10.1016/j.soilbio.2016.06.020

Soong, J.L., Reuss, D., Pinney, C., Boyack, T., Haddix, M.L., Stewart, C.E., Cotrufo, M.F., 2014. Design and operation of a continuous 13C and 15N labeling chamber for uniform or differential, metabolic and structural, plant isotope labeling. JoVE (Journal of Visualized Experiments) e51117. doi:10.3791/51117

Soong, J.L., Vandegehuchte, M.L., Horton, A.J., Nielsen, U.N., Denef, K., Shaw, E.A., de Tomasel, C.M., Parton, W., Wall, D.H., Cotrufo, M.F., 2016. Soil microarthropods support ecosystem productivity and soil C accrual: Evidence from a litter decomposition study in the tallgrass prairie. Soil Biology and Biochemistry 92, 230–238. doi:10.1016/j.soilbio.2015.10.014

Thakur, M.P., Geisen, S., 2019. Trophic Regulations of the Soil Microbiome. Trends in Microbiology 27, 771–780. doi:10.1016/j.tim.2019.04.008

Trap, J., Bonkowski, M., Plassard, C., Villenave, C., Blanchart, E., 2016. Ecological importance of soil bacterivores for ecosystem functions. Plant and Soil 398, 1–24. doi:10.1007/s11104-015-2671-6

van den Hoogen, J., Geisen, S., Wall, D.H., Wardle, D.A., Traunspurger, W., de Goede, R.G.M., Adams, B.J., Ahmad, W., Ferris, H., Bardgett, R.D., Bonkowski, M., Campos-Herrera, R., Cares, J.E., Caruso, T., de Brito Caixeta, L., Chen, X., Costa, S.R., Creamer, R., da Cunha e Castro, J.M., Dam, M., Djigal, D., Escuer, M., Griffiths, B.S., Gutiérrez, C., Hohberg, K., Kalinkina, D., Kardol, P., Kergunteuil, A., Korthals, G., Krashevska, V., Kudrin, A.A., Li, Q., Liang, W., Magilton, M., Marais, M., Martín, J.A.R., Matveeva, E., Mayad, E.H., Mzough, E., Mulder, C., Mullin, P., Neilson, R., Nguyen, T.A.D., Nielsen, U.N., Okada, H., Rius, J.E.P., Pan, K., Peneva, V., Pellissier, L., da Silva, J.C.P., Pitteloud, C., Powers, T.O., Powers, K., Quist, C.W., Rasmann, S., Moreno, S.S., Scheu, S., Setälä, H., Sushchuk, A., Tiunov, A.V., Trap, J., Vestergård, M., Villenave, C., Waeyenberge, L., Wilschut, R.A., Wright, D.G., Keith, A.M., Yang, J., Schmidt, O., Bouharroud, R., Ferji, Z., van der Putten, W.H., Routh, D., Crowther, T.W., 2020. A global database of soil nematode abundance and functional group composition. Scientific Data 7, 103. doi:10.1038/s41597-020-0437-3

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707. doi:10.1016/0038-0717(87)90052-6

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633. doi:10.1126/science.1094875

Wickham, H., François, R., Henry, L., Müller, K., RStudio, 2019. dplyr: A grammar of data manipulation.

Wickings, K., Grandy, A.S., 2011. The oribatid mite Scheloribates moestus (Acari: Oribatida) alters litter chemistry and nutrient cycling during decomposition. Soil Biology and Biochemistry 43, 351–358. doi:10.1016/j.soilbio.2010.10.023

Xie, L., Yan, Y., Zhang, Z.-Q., 2018. Development, survival and reproduction of Stratiolaelaps scimitus (Acari: Laelapidae) on four diets. Systematic and Applied Acarology 23, 779–794. doi:10.11158/saa.23.4.16

Yang, S.-H., Wang, D., Chen, C., Xu, C.-L., Xie, H., 2020. Evaluation of Stratiolaelaps scimitus (Acari: Laelapidae) for controlling the root-knot nematode, Meloidogyne incognita (Tylenchida: Heteroderidae). Scientific Reports 10, 5645. doi:10.1038/s41598-020-62643-2

Zeileis, A., Grothendieck, G., Ryan, J.A., Ulrich, J.M., Andrews, F., 2023. zoo: S3 Infrastructure for Regular and Irregular Time Series (Z's Ordered Observations).

Zheng, J., Dini-Andreote, F., Luan, L., Geisen, S., Xue, J., Li, H., Sun, B., Jiang, Y., 2022. Nematode predation and competitive interactions affect microbe-mediated phosphorus dynamics. MBio 13, e03293-21. doi:10.1128/mbio.03293-21

Zhu, X., Jackson, R.D., DeLucia, E.H., Tiedje, J.M., Liang, C., 2020. The soil microbial carbon pump: From conceptual insights to empirical assessments. Global Change Biology 26, 6032–6039. doi:10.1111/gcb.15319

# CHAPTER 6: CONCLUSION

This dissertation investigates the complex impacts of regenerative agriculture on SOM dynamics and soil fauna biodiversity, encompassing a broad range of objectives from global patterns and policy needs to mechanistic understanding. The first three chapters contribute to a holistic understanding of how regenerative practices influence soil health, carbon sequestration, and biodiversity, highlighting their potential for sustainable agricultural systems. The dissertation concludes with a fourth chapter presenting a detailed examination of the biological mechanisms underlying MAOM formation through a highly controlled lab experiment.

Chapter 2 presents a global meta-analysis demonstrating that regenerative practices, including no-till and cropping system intensification, significantly increase both POM and MAOM. These findings emphasize the importance of adopting synergistic practices, such as integrated crop-livestock (ICL) systems, to maximize soil carbon storage and improve soil health. The study highlights specific knowledge gaps, including the need for a deeper understanding of the mechanisms driving these synergies, which are essential for optimizing regenerative management strategies to mitigate climate change and enhance ecosystem services.

Chapter 3 shifts focus to the United States, evaluating the impacts of diversified agricultural systems on soil carbon, health, and yield. The policy brief indicate the importance of long-term implementation of multiple regenerative practices to achieve consistent positive outcomes. The findings advocate for increased funding, longer participation in conservation programs, and regionally tailored conservation efforts. This chapter provides empirical evidence to inform policy decisions and support the widespread adoption of regenerative agricultural practices.

Chapter 4 delves into the field level, examining the effects of varying degrees of regenerative practice adoption on SOM dynamics and soil fauna biodiversity within the Cheney Watershed, Kansas.

By developing a Regenerative Farming Index (RFI), the study quantifies the extent of regenerative practices and links higher RFI scores to increased carbon and nitrogen stocks in both POM and MAOM. The positive correlation between RFI and soil fauna diversity metrics highlights the potential of regenerative agriculture to foster biodiversity and enhance soil health, while also speculating on the mechanisms by which specific practices influence both pools. Path analysis reveals the indirect influence of soil fauna on SOM through their role in enhancing regenerative practices.

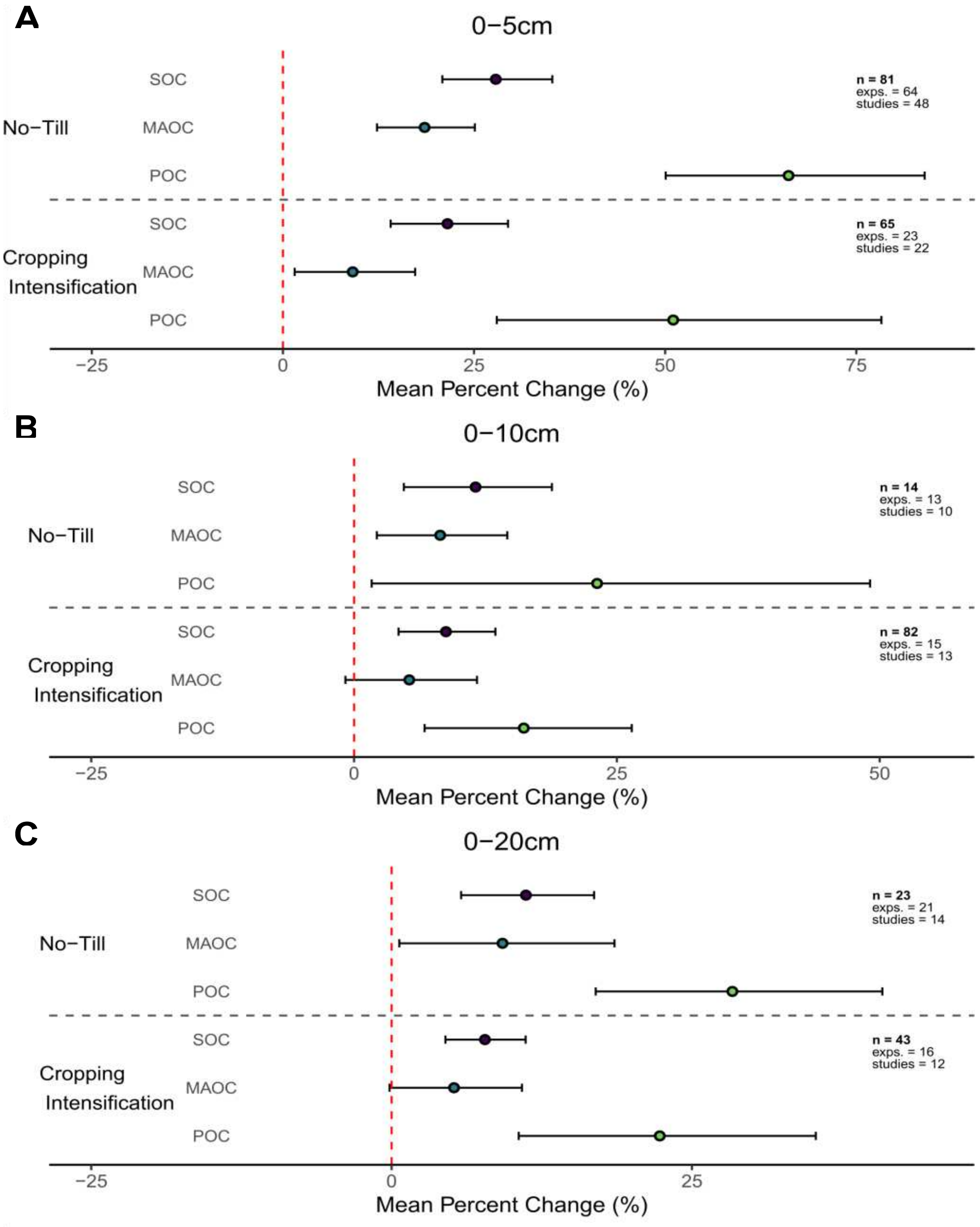
Finally, Chapter 5 explores the mechanistic interactions between soil fauna and SOM, particularly focusing on the roles of bacterivorous nematodes and predatory mites. The study elucidates the complex interplay between these organisms in shaping microbial necromass accrual and SOM dynamics. The findings highlight the critical role of the soil food web in mediating carbon and nitrogen dynamics, providing insights into the biological underpinnings of SOM stabilization. This nuanced understanding emphasizes the potential of considering the full soil food web in biological interventions to enhance soil health and carbon sequestration.

Overall, this dissertation advances the understanding of SOM dynamics and soil fauna biodiversity under regenerative agriculture, providing valuable insights for sustainable soil management and climate change mitigation. By integrating global and local scales, it offers a comprehensive view of how regenerative practices can restore soil health and sequester carbon, contributing to more resilient and productive agricultural systems. The findings emphasize the importance of adopting a systems-level approach to regenerative agriculture, considering the synergistic effects of multiple practices to achieve the greatest benefits for the environment and agricultural productivity.

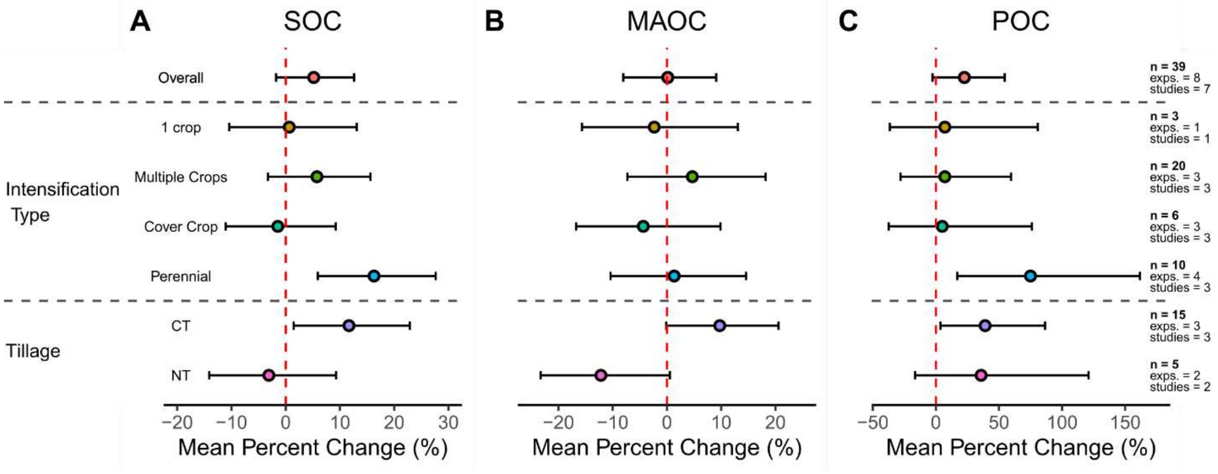
# APPENDIX

# 1. SUPPLEMENTARY FILE FOR CHAPTER 2

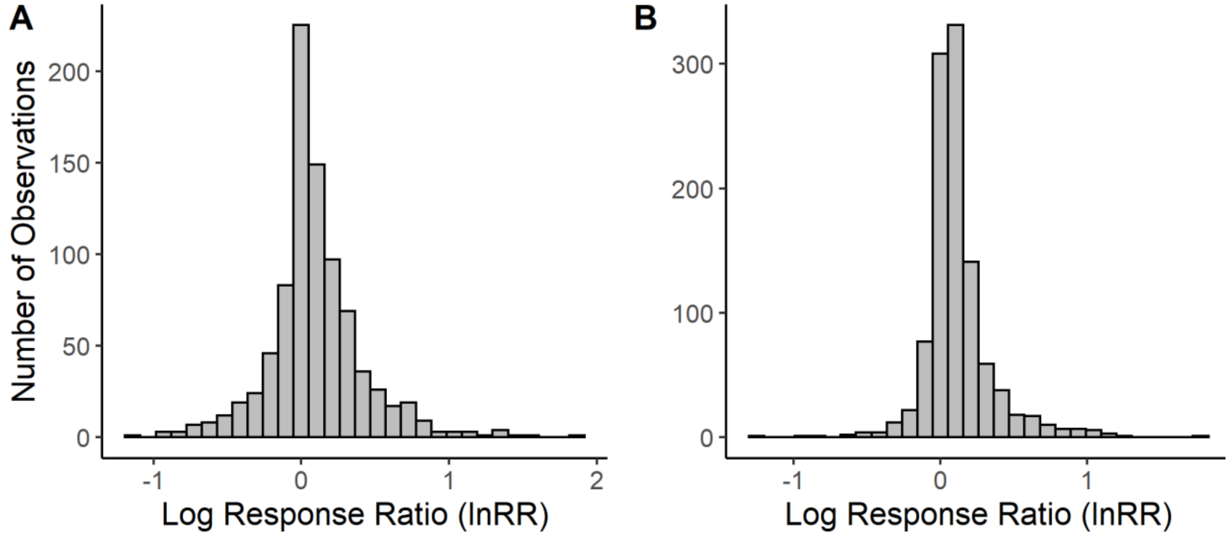
## *1.1 Figures*



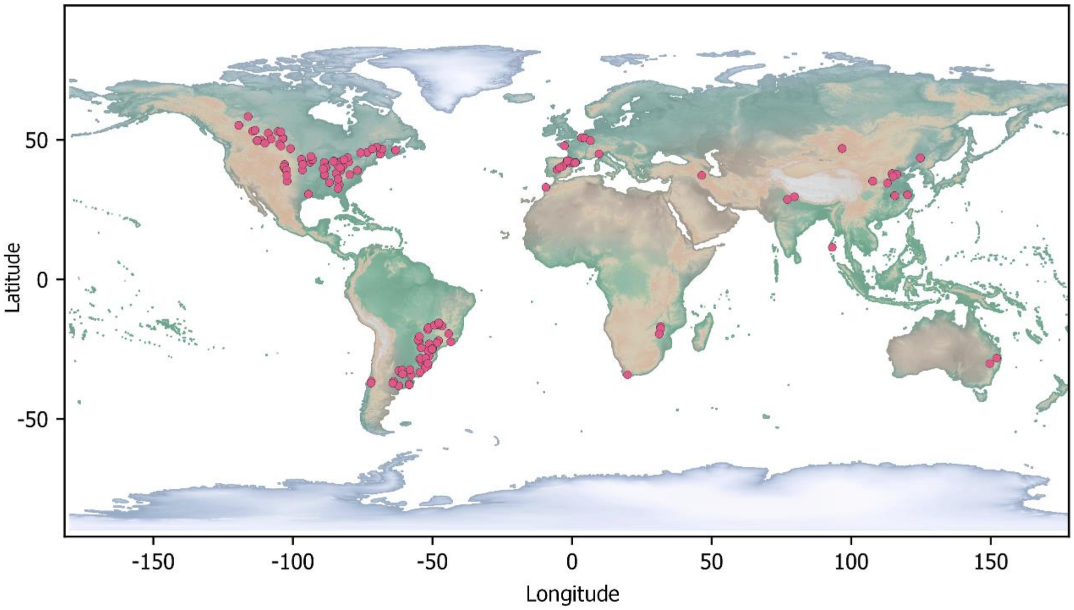
**Supporting Figure 2.1. Overall response of carbon concentrations** (g C kg soil$^{-1}$) in total soil organic carbon (SOC), mineral-associated organic carbon (MAOC), and particulate organic carbon (POC) to regenerative agricultural management in 0-5 cm (**A**), 0-10 cm (**B**), and 0-20 cm (**C**) soil layers. No-Till systems are compared to conventional tillage systems. Cropping Intensification includes i) eliminating summer fallow in monocrop systems, ii) increasing the number of annual crops grown per year, iii) planting cover crops, and iv) incorporating perennial crops in rotation. Smaller depth increments were not aggregated by calculating weighted means, only exact depth increments were included. Effect sizes were considered significant if 95% confidence interval did not overlap zero.



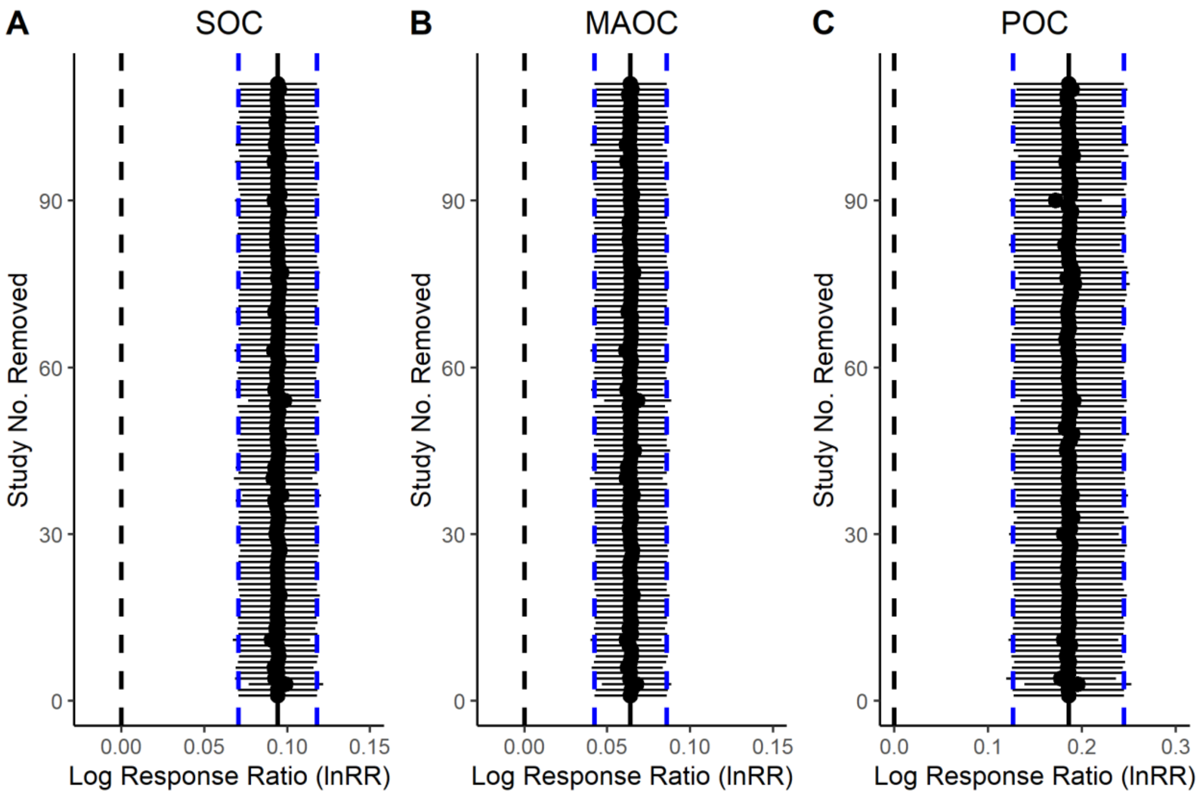
**Supporting Figure 2.2. Effect of cropping intensification on subsoil** (20-100cm) carbon concentrations (g C kg soil$^{-1}$) in total soil organic carbon (**A**; SOC), mineral-associated organic carbon (**B**; MAOC), and particulate organic carbon (**C**; POC) as moderated by experimental duration, type of cropping intensity, rotation diversity, and tillage management. Tillage management, residue management, fertilization regime, and environmental variables held constant. 'Intensification Type' signifies type of intensification (i.e. '1 crop' = intensification of crop-fallow to rotation of 1 annual cash crop per year, 'Multiple Crops' = intensification of 1 cash crop per year to multiple cash crops per year, 'Cover Crop' = intensification of annual cash crop system to annual rotation + cover crop, 'Perennial' = intensification of annual cropping system to system with a perennial crop included in rotation). Effect sizes were considered significant if 95% confidence interval did not overlap zero. The number of observations (n), experiments (exps.), and studies analyzed for each moderator variable is displayed on the far right of figure.



**Supporting Figure 2.3:** Publication bias across the 648 observations in the Tillage (**A**) and Cropping System Intensification (**B**) data sets. A positive lnRR indicates that regenerative management increased soil organic carbon (SOC), mineral-associated organic carbon (MAOC), or particulate organic carbon (POC) to a depth of 0–100 cm relative to a conventional control. Left or right skew indicates evidence of publication bias.



**Supporting Figure 2.4:** Map of experimental studies



**Supporting Figure 2.5:** The results of the sensitivity analysis, which was completed using a Jacknife technique. The solid black line represents the overall lnRR and the blue dashed lines the lower and upper 95% confidence interval of the overall lnRR (prior to publication removal). The solid black line represents a null effect. Recomputed lnRR and 95% confidence intervals that do not cross zero were considered significant.

## *1.2 Tables*

**Supporting Table 2.6**: Table of all moderators tested for the effect of no-till (NT) compared to conventional tillage on soil organic carbon (SOC), mineral-associated organic carbon (MAOC), and particulate organic carbon (POC).The abbreviations are mean annual precipitation minus potential evapotranspiration (MAP–PET) and mean annual temperature (MAT).

| Moderator levels | n* | df† | *Q*‡ | *p* value§ | lnRR# | se◊ | ci.lb¶ |
|---|---|---|---|---|---|---|---|
| **SOC** | | | | | | | |
| Experimental Duration | 235 | 2 | 1.764 | 0.115 | | | |
| <6 yr | 27 | 232 | | | 0.026 | 0.042 | -0.057 |
| 6-12 yr | 82 | 232 | | | 0.114 | 0.023 | 0.069 |
| >12 yr | 126 | 232 | | | 0.122 | 0.019 | 0.084 |
| Tillage Frequency | 234 | 1 | 3.04 | 0.083 | | | |
| once | 143 | 232 | | | 0.089 | 0.019 | 0.052 |
| multiple | 91 | 232 | | | 0.136 | 0.023 | 0.091 |
| Cropping Intensity | 235 | 2 | 8.866 | **< 0.001** | | | |
| 1 crop | 153 | 232 | | | 0.129 | 0.017 | 0.096 |
| multiple crops | 26 | 232 | | | 0.059 | 0.025 | 0.011 |
| cover crop | 56 | 232 | | | 0.066 | 0.019 | 0.029 |
| MAP – PET (mm yr$^{-1}$) | 235 | 1 | 1.764 | 0.184 | 0.000 | 0.000 | 0.000 |
| Soil pH | 164 | 1 | 0.368 | 0.544 | 0.015 | 0.003 | 0.009 |
| MAT (°C) | 235 | 1 | 6.356 | **0.012** | 0.008 | 0.001 | 0.006 |
| Sand Content (%) | 182 | 1 | 0.002 | 0.964 | 0.002 | 0.000 | 0.002 |
| **MAOC** | | | | | | | |
| Experimental Duration | 235 | 2 | 1.795 | 0.168 | | | |
| <6 yr | 27 | 232 | | | 0.014 | 0.038 | -0.06 |
| 6-12 yr | 82 | 232 | | | 0.088 | 0.021 | 0.047 |
| >12 yr | 126 | 232 | | | 0.092 | 0.018 | 0.058 |
| Tillage Frequency | 234 | 1 | 13.835 | **< 0.001** | | | |
| once | 143 | 232 | | | 0.049 | 0.017 | 0.016 |
| multiple | 91 | 232 | | | 0.133 | 0.02 | 0.094 |
| Cropping Intensity | 235 | 2 | 5.281 | **0.006** | | | |
| 1 crop | 153 | 232 | | | 0.078 | 0.015 | 0.049 |
| multiple crops | 26 | 232 | | | 0.125 | 0.022 | 0.08 |
| cover crop | 56 | 232 | | | 0.07 | 0.018 | 0.035 |
| MAP – PET (mm yr$^{-1}$) | 235 | 1 | 1.24 | 0.265 | 0.000 | 0.000 | 0.000 |
| Soil pH | 164 | 1 | 0.076 | 0.783 | 0.01 | 0.002 | 0.006 |
| MAT (°C) | 235 | 1 | 4.94 | **0.026** | 0.006 | 0.001 | 0.004 |
| Sand Content (%) | 182 | 1 | 0.448 | 0.503 | 0.002 | 0.000 | 0.002 |
| **POC** | | | | | | | |
| Experimental Duration | 235 | 2 | 0.812 | 0.445 | | | |
| <6 yr | 27 | 232 | | | 0.067 | 0.095 | -0.119 |
| 6-12 yr | 82 | 232 | | | 0.189 | 0.052 | 0.086 |
| >12 yr | 126 | 232 | | | 0.199 | 0.044 | 0.113 |
| Tillage Frequency | 234 | 1 | 0.128 | 0.72 | | | |
| once | 143 | 232 | | | 0.169 | 0.041 | 0.088 |

**Supporting Table 2.7**: Table of all moderators tested for the effect of cropping system intensification on soil organic carbon (SOC), mineral-associated organic carbon (MAOC), and particulate organic carbon (POC).The abbreviations are no-till (NT), conventional tillage (CT), mean annual precipitation minus potential evapotranspiration (MAP–PET) and mean annual temperature (MAT).

| Moderator levels | n* | df† | *Q*‡ | *p* value§ | lnRR# | se◊ | ci.lb¶ |
|---|---|---|---|---|---|---|---|
| **SOC** | | | | | | | |
| Experimental Duration | 319 | 2 | 6.834 | **0.001** | | | |
| <6 yr | 63 | 316 | | | 0.053 | 0.031 | -0.007 |
| 6-12 yr | 151 | 316 | | | 0.116 | 0.018 | 0.081 |
| >12 yr | 105 | 316 | | | 0.162 | 0.019 | 0.125 |
| Intensification Type | 319 | 3 | 28.283 | **<0.001** | | | |
| 1 crop | 89 | 315 | | | 0.131 | 0.018 | 0.096 |
| multiple crops | 42 | 315 | | | 0.002 | 0.021 | -0.039 |
| cover crop | 90 | 315 | | | 0.121 | 0.017 | 0.087 |
| perennial | 98 | 315 | | | 0.15 | 0.017 | 0.116 |
| Diversity | 319 | 2 | 18.279 | **<0.001** | | | |
| monoculture | 102 | 316 | | | 0.129 | 0.015 | 0.099 |
| biculture | 145 | 316 | | | 0.116 | 0.015 | 0.086 |
| polyculture | 72 | 316 | | | 0.107 | 0.015 | 0.077 |
| Cover Crop Functional Grp | 95 | 2 | 0.349 | 0.706 | | | |
| grass | 34 | 92 | | | 0.093 | 0.021 | 0.051 |
| legume | 43 | 92 | | | 0.109 | 0.02 | 0.069 |
| mix | 18 | 92 | | | 0.099 | 0.023 | 0.053 |
| Tillage | 270 | 1 | 4.084 | **<0.001** | | | |
| CT | 109 | 317 | | | 0.149 | 0.017 | 0.115 |
| NT | 161 | 317 | | | 0.098 | 0.017 | 0.065 |
| MAP – PET (mm yr$^{-1}$) | 301 | 1 | 1.041 | 0.308 | 0.000 | 0.000 | 0.000 |
| Soil pH | 197 | 1 | 0.32 | 0.571 | 0.016 | 0.002 | 0.011 |
| MAT (°C) | 319 | 1 | 1.247 | 0.264 | 0.008 | 0.001 | 0.006 |
| Sand Content (%) | 191 | 1 | 6.119 | **0.013** | 0.004 | 0.000 | 0.003 |
| **MAOC** | | | | | | | |
| Experimental Duration | 319 | 2 | 3.917 | **0.021** | | | |
| <6 yr | 63 | 316 | | | 0.032 | 0.03 | -0.028 |
| 6-12 yr | 151 | 316 | | | 0.063 | 0.017 | 0.028 |
| >12 yr | 105 | 316 | | | 0.103 | 0.019 | 0.066 |
| Intensification Type | 319 | 3 | 2.045 | 0.107 | | | |
| 1 crop | 89 | 315 | | | 0.051 | 0.016 | 0.02 |
| multiple crops | 42 | 315 | | | 0.085 | 0.019 | 0.048 |
| cover crop | 90 | 315 | | | 0.071 | 0.015 | 0.041 |
| perennial | 98 | 315 | | | 0.072 | 0.015 | 0.042 |
| Diversity | 319 | 2 | 16.432 | **<0.001** | | | |
| monoculture | 102 | 316 | | | 0.095 | 0.016 | 0.064 |
| biculture | 145 | 316 | | | 0.066 | 0.015 | 0.036 |
| polyculture | 72 | 316 | | | 0.046 | 0.016 | 0.015 |