DISSERTATION

COVER CROPS FOR ECOLOGICAL MANAGEMENT OF U.S. AGRICULTURAL SYSTEMS: QUANTIFYING ECOSYSTEM SERVICES ACROSS MULTIPLE SCALES

Submitted by

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ABSTRACT

COVER CROPS FOR ECOLOGICAL MANAGEMENT OF U.S. AGRICULTURAL SYSTEMS: QUANTIFYING ECOSYSTEM SERVICES ACROSS MULTIPLE SCALES

Managing agricultural systems to provide multiple ecosystem services (ES) beyond food provisioning has gained considerable attention in recent years. The integration of cover crops (CC) into U.S. cropping systems presents an opportunity to support multifunctional agricultural systems, which alleviate negative environmental impacts of agriculture, mitigate greenhouse gas (GHG) emissions and support sustained crop production. However, CC impacts on these ES are variable and depend on management and site characteristics, contributing to uncertainty surrounding to what extent CC can improve ES. Reducing this uncertainty is critical to both identify appropriate environmental and management conditions for CC adoption and improve the estimated potential for CC to improve multifunctionality of U.S. cropping systems. This dissertation aims to quantify CC impacts on ES at multiple scales, exploring benefits to the soil microbiome, at the farm level, and nationally. Throughout this assessment I explore how these effects are influenced by climate and soil characteristics and how management can be leveraged to optimize the provision of ES.

Chapter two estimates the potential for widespread adoption of CC to increase soil organic carbon (C) stocks and mitigate GHG emissions in the U.S. Analysis using current U.S. crop management data and a biogeochemical model revealed that the mitigation potential over a 20 year period is lower than previous estimates due to regional variability, decreasing rates of C accrual over time, and limited CC integration. Changes in N_2O emissions did not offset C sequestration but introduced large uncertainty surrounding total national mitigation potential. Soil C gains due

to CC offer important co-benefits to U.S. cropping systems, but the contribution of CC to achieving U.S. emissions targets will likely be lower than previously anticipated. Our spatially-explicit analysis also highlights regions where adoption of CC can have greater relative contributions to GHG mitigation.

I then quantify a larger suite of ES in dryland wheat systems of the semi-arid western U.S., a particularly challenging context for CC due to lower potential productivity and associated economic trade-offs. I used two existing field trials to monitor CC impacts on soil health, cash crop productivity, and economics over a period of six years. No-till, CC planting window, and the sale of CC biomass as forage were also explored as strategies to optimize ES provision and economic viability. Chapters three and four demonstrate that the integration of CC amidst water limitations can benefit erosion control and soil structure, but also present significant productivity and economic trade-offs. The integration of fall-planted CC, no-till management, and the use of CC for forage provided the greatest potential for maximizing ES benefits in an economically viable manner.

In Chapter five, I conducted a greenhouse study to examine the impact of CC type and functional diversity on microbial community composition and associated ES. Plant functional types (Poaceae, Brassicaceae, and Fabaceae) were associated with distinct increases in ES proxies, which appear to be mediated by shifts in microbial community composition. Specifically, Fabaceae (legume) CC enhanced the presence of copiotrophic microbes, which were associated with improvements in soil structure and high enzyme activity, a proxy for nutrient cycling. Poaceae and Brassicaceae led to improvements in microbial diversity. Ecosystem service benefits and microbial community shifts were conserved in diverse CC mixtures, contributing to increased multifunctionality.

Across studies and scales, CC were observed to support a number of ES that address environmental concerns resulting from modern intensive agricultural practices. However, slight benefits and substantial productivity trade-offs in water-limited systems may limit the extent to which CC can mitigate GHG emissions and restore soil C reserves nationally. Management choices, such as CC composition and diversity, no-till management, and the sale of a portion of CC biomass as forage, can be leveraged to optimize the provision of ES in an economically viable manner. Overall, CC effectively contribute to multifunctional agroecosystems whose ES extend beyond food provisioning.

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CHAPTER 1: INTRODUCTION

Agricultural systems provide many ecosystem services (ES) that are critical for the health of the environment and society, including climate regulation, air and water purification, and maintenance of soil fertility (Millennium Ecosystem Assessment, 2005; Bennett & Balvanera, 2007). However, the advent of chemical fertilizer and herbicides, combined with pressure to feed a rapidly growing population led to a shift in agricultural practices during the 20th century, with a more narrow focus on food provisioning (Gliessman, 2006). Management practices that replenish soil carbon (C) and organic nutrients in the soil were replaced with a reliance on chemical inputs and simplified cropping systems (Auclair, 1976; Tilman, 1999). As a result, increases in the global food supply were accompanied by inadvertent, detrimental impacts to the environment and multiple ES (Tilman et al., 2001). These impacts are widespread and burden society with costs to mitigate air and water pollution, greenhouse gas emissions, biodiversity losses, among others.

To alleviate these environmental impacts while sustaining crop production, agricultural systems must support multiple ES and leverage internal, biologically-driven processes to reduce external inputs (Tilman, 1999; Drinkwater & Snapp, 2007; Robertson & Swinton, 2005). The Natural Resources Conservation Service defined four basic management principles that can support this goal: 1) minimize soil disturbance; 2) retain soil cover; 3) maximize biodiversity; and 4) maximize the presence of living roots in the soil (Natural Resources Conservation Service, 2021). The use of cover crops (CC), or crops that replace fallow periods and are not sold for profit, integrates three of these four principles and has been shown to support numerous ES at multiple scales (Fig. 1.1; Snapp et al., 2005; Schipanski et al., 2014).

At the farm-level, CC support numerous ES which promote stable crop production and reduce the need for chemical inputs (Fig. 1.1; Snapp et al., 2005; Daryanto et al., 2018). Replacing bare fallow with CC can control erosion, thereby mitigating potential productivity losses over time (Kaspar & Singer, 2011; Miguez et al., 2016). Cover crops can also reduce the need for herbicides and pesticides due to their pest and weed suppression benefits (Haramoto & Gallandt, 2007; Snapp et al., 2005). Nutrient-use-efficiency is also improved with CC, as increased C inputs and N assimilation reduce nutrient losses and increase soil C stocks (Tonitto et al., 2006; Kramberger et al., 2009; Daryanto et al., 2018).

Relatively recent advances in microbial sequencing technologies have demonstrated the many CC benefits on microbial communities, which also contribute to ES benefits observed on-farm (Fig. 1.1; Lehman et al., 2015). Increased and consistent C inputs from CC can help reverse losses in microbial community abundance and activity that have resulted from modern agricultural practices (Muhammad et al., 2021; Kim et al., 2020). Cover crops also have notable effects on microbial community composition (Finney, Buyer & Kaye, 2017; Martinez-Garcia et al., 2018); CC residue retention has been shown to promote microbial diversity, which can be associated with the functional capacity and resilience of microbial communities (Nevins et al., 2018; Lehman et al., 2015). Active microbial communities also promote improvements in soil structure and enhance the potential for nutrient cycling (Bronick & Lal, 2005; Stark & Condron, 2008). Due to these numerous ES benefits, CC are widely recognized as a cost-effective strategy to improve agroecosystem multifunctionality (Keestra et al., 2018).

Ecosystem services provided by CC extend beyond the farm to improve air and water quality, offset GHG emissions, and maintain productivity of agricultural lands (Fig. 1.1; Daryanto et al., 2018; Schipanski et al., 2014). To maximize these public benefits, U.S. conservation programs have encouraged CC adoption since the 1930s (Kell & McKee, 1936). These programs initially targeted erosion control benefits of CC following the devastating environmental impacts of the Dust Bowl (Kell & McKee, 1936; Natural Resources Conservation Service, 2022; Hartwig & Ammon, 2002). Concern for water quality and non-point source pollution in the 1970s led to further CC incentives (Carpenter et al., 1998; National Research Council, 1993), as CC can effectively reduce nutrient losses from leaching and runoff (Kaspar et al., 2012; Kladivko et al., 2004). Recently the ES supported by CC are of particular interest due to soil C accrual benefits that can offset GHG emissions (Kaye & Quemada, 2017; Bossio et al., 2020). Cover crops can increase the land C sink in the U.S. substantially, with estimates as high as 103 Mt CO_2e year^{-1}, and their adoption is an integral part of proposed scenarios to meet national emissions targets (Fargione et al., 2018; United States of America, 2021). Since 2021, the Natural Resource Conservation Service (NRCS) has drastically expanded funding for CC incentives, launching the Pandemic Cover Crop Program, a new Cover Crop Initiative through the Environmental Quality Incentives Program, and the Climate Smart Commodities Program (U.S. Department of Agriculture, 2022).

The numerous co-benefits of CC suggest that they can effectively support stable, resilient crop production, while combatting some of the greatest environmental threats facing modern society. However, the extent to which CC improve ES is highly variable and can depend on climate, soil characteristics and management choices (Daryanto et al., 2018; McClelland et al., 2021). Ecosystem services such as weed suppression, C accrual and erosion control are well correlated with CC biomass production (Finney, White & Kaye, 2016), which is sensitive to annual precipitation and mean temperatures (Ruis et al., 2019). In many cases, CC result in in trade-offs among ES; for example, in water-limited systems CC can compete with cash crops for water and lead to cash crop yield penalties (Wang et al., 2021; Eash et al., 2021).

Management of CC can also be tailored to target certain ES. For example, selection of key CC species or functional groups can favor particular ES; grass CC have been shown to contribute to improved N retention, while the N-fixation capability of legumes can contribute to improved cash crop productivity (Finney, White and Kaye, 2016; Quemada et al., 2020). Tillage regime can also have interactive effects with CC to influence ES. No-till can improve CC biomass production in water-limited systems due to increased water capture and retention (Peterson et al., 2019). Soil structure improvements under no-till can also enhance C gains due to CC (Olson et al., 2014; Büchi et al., 2018). Understanding variability in ES due to CC among different environmental and management contexts is critical to increase certainty surrounding CC outcomes, balance ES trade-offs, and optimize associated management practices.

In this dissertation, I assess the extent to which CC can provide ES that support stable crop production, alleviate environmental contamination due to agriculture and reduce atmospheric GHG concentrations in the U.S. Central to this objective is understanding how environmental context and management choices contribute to variability in ES provisioning due to CC. I conducted studies in the greenhouse, field and using modelled data to quantify CC impacts on ES in the soil microbiome, at the farm-level, and nationally. In all studies, management options were explored to optimize ES trade-offs and benefits.

This dissertation begins by quantifying ES provisioning due to widespread CC adoption at the national scale, focusing on soil organic C accrual due to its numerous co-benefits and its relevance for GHG mitigation. To date, the estimated net C sequestration potential of CC in the U.S. varies widely despite growing national investment (Sperow et al., 2003; Fargione et al., 2018;

Roe et al., 2021). Previous estimates have not considered fine-scale variability in environmental conditions, the influence of current U.S. agricultural practices, or potential N_2O trade-offs that may offset GHG benefits of C sequestration (Lugato et al., 2018; Basche et al., 2014). A process-based model and current U.S. crop management data were used to meet the following objectives: 1) quantify soil organic C accrual due to CC under high adoption scenarios, 2) examine N_2O trade-offs, and 3) understand how climate, soil and management factors contribute to rates of C accrual and GHG mitigation.

In Chapters three and four, I quantify a larger suite of ES at the farm-scale in semi-arid, dryland wheat-fallow systems on the Colorado Plateau. Water limitations present a particularly challenging context for CC adoption in this region, as environmental benefits can be associated with significant cash crop yield penalties (Wang et al., 2021; Eash et al., 2021). However, as management priorities shift to prioritize multiple ES, local producers were interested in assessing the viability of CC to address widespread soil degradation and erosion. Furthermore, semi-arid, dryland cropping systems also constitute a substantial portion of U.S. cropland (Hansen et al., 2016), so evaluating trade-offs using empirical data in these systems is critical to assessing the potential ES due to CC nationally. I sampled two existing field trials to quantify the impact of CC on soil health, cash crop productivity and farm economics. No-till and CC planting window were both explored as potential levers to alleviate competition for water, while the sale of a portion of CC biomass as forage was considered as a hypothetical means to compensate for potential reductions in net profit. Chapter three focuses on short-term impacts on cash crop productivity and associated metrics (water and N availability) due to CC. Chapter four is an interdisciplinary, systems-level analysis of CC impacts on multiple ES and farm economics over a six-year period.

Finally, I conducted a greenhouse study to assess the impact of CC functional groups and diversity on microbial community composition and associated ES. The diversity of CC mixtures has been shown to alleviate ES trade-offs among CC species, particularly when species with varying functional attributes are included (Finney & Kaye, 2016). Furthermore, as individual CC types can differentially affect both ES and microbial community composition, CC offer a useful opportunity to study linkages between plants, microbes, and ES (Wick et al., 2017; Martinez-Garcia et al., 2018; Lehman et al., 2015). The objectives of Chapter five were to: 1) determine the effect of CC functional composition on microbially-derived ES; 2) explore relationships between CC functional type, CC microbial community composition, and microbially-derived ES; and 3) quantify ES trade-offs and multifunctionality in diverse CC mixtures.

The ES benefits of CC occur at multiple scales and are well-documented, suggesting a high potential for CC to support the multifunctionality if agricultural systems. The work presented here will consider differences in environmental and management contexts to better inform multiple stakeholders about likely ES outcomes at multiple scales. Overall, findings from this dissertation will inform optimal CC management, appropriate environmental conditions for CC adoption, and the estimated potential for CC to improve multifunctionality of U.S. cropping systems.

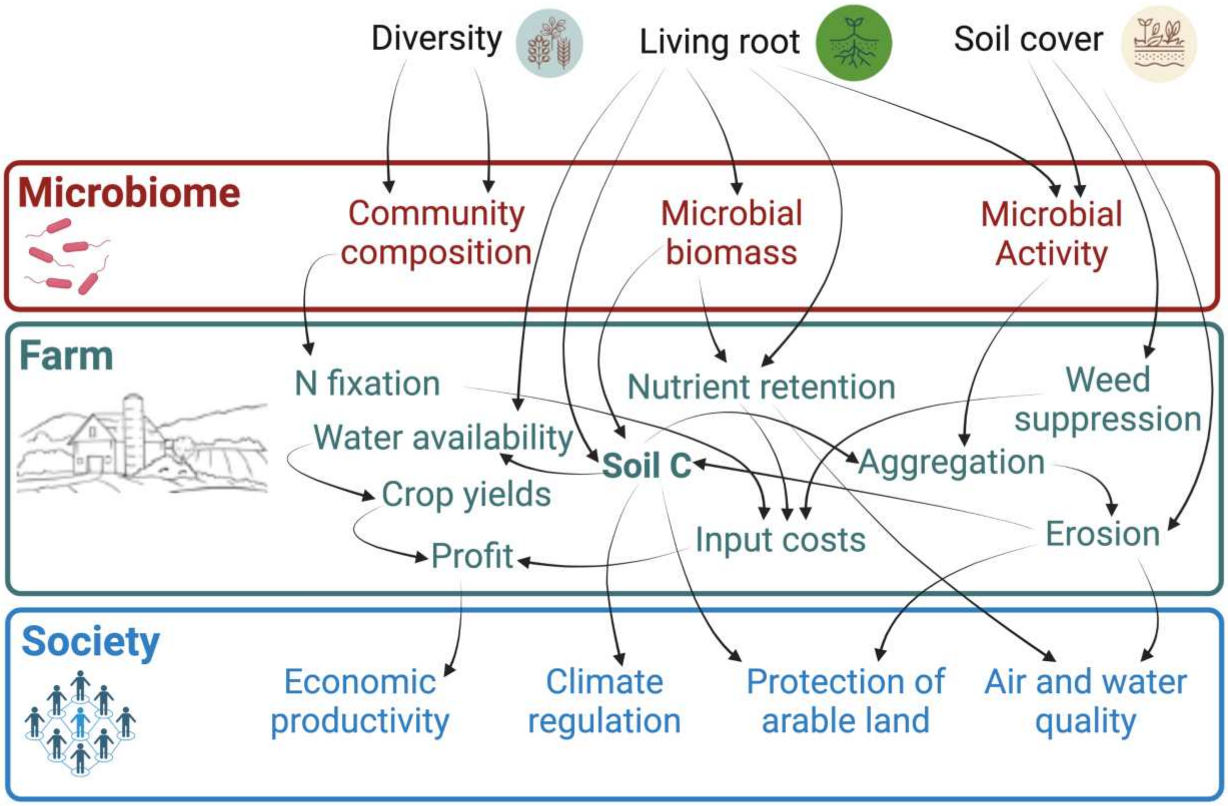

Figure 1.1. Key ecosystem services and associated metrics that can be influenced by cover crops and are observed at different scales (society, farm and microbiome). Arrows indicate potential interactions among metrics.

REFERENCES

Auclair, A. N. (1976). Ecological factors in the development of intensive management ecosystems in the midwestern USA. *Ecology, 57*, 431-444.

Basche, A. D., Miguez, F. E., Kaspar, T. C., & Castellano, M. J. (2014). Do cover crops increase or decrease nitrous oxide emissions? a meta-analysis. *Journal of Soil and Water Conservation, 69*, 471–482. https://doi.org/10.2489/jswc.69.6.471

Bennett, E.M., & Balvanera, P.A. (2007). The future of production systems: challenges and opportunities in a globalized world. *Frontiers in Ecology and the Environment, 5*, 191–198. https://doi.org/10.1890/1540-9295(2007)5[191:TFOPSI]2.0.CO;2

Bossio, D. A., Cook-Patton, S. C., Ellis, P. W., Fargione, J., Sanderman, J., Smith, P., Wood, S., Zomer, R. J., Von Unger, M., Emmer, I. M., & Griscom, B. W. (2020). The role of soil carbon in natural climate solutions. *Nature Sustainability*, *3*(5), 391-398. https://doi.org/10.1038/s41893-020-0491-z

Bronick, C. J., & Lal, R. (2005). Soil structure and management: A review. *Geoderma*, *124*(1–2), 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005

Büchi, L., Wendling, M., Amossé, C., Necpalova, M., & Charles, R. (2018). Importance of cover crops in alleviating negative effects of reduced soil tillage and promoting soil fertility in a winter wheat cropping system. *Agriculture, Ecosystems & Environment*, *256*, 92-104. https://doi.org/10.1016/j.agee.2018.01.005

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological*

Applications, *8,* 559-568. https://doi.org/10.1890/1051-0761(1998)008[0559:NPOSWW]2.0.CO;2

Daryanto, S., Fu, B., Wang, L., Jacinthe, P. A., & Zhao, W. (2018). Quantitative synthesis on the ecosystem services of cover crops. *Earth-Science Reviews*, *185*, 357-373. https://doi.org/10.1016/j.earscirev.2018.06.013

Drinkwater, L. E., & Snapp, S. S. (2007). Nutrients in agroecosystems: Rethinking the management paradigm. *Advances in Agronomy, 92,* 163-186. https://doi.org/10.1016/S0065-2113(04)92003-2

Eash, L., Berrada, A. F., Russell, K., & Fonte, S. J. (2021). Cover crop impacts on water dynamics and yields in dryland wheat systems on the Colorado Plateau. *Agronomy*, *11*(6), 1102. https://doi.org/10.3390/agronomy11061102

Fargione, J. E., Bassett, S., Boucher, T., Bridgham, S. D., Conant, R. T., Cook-Patton, S. C., Ellis, P. W., Falcucci, A., Fourqurean, J. W., Gopalakrishna, T., Gu, H., Henderson, B., Hurteau, M. D., Kroeger, K. D., Kroeger, T., Lark, T. J., Leavitt, S. M., Lomax, G., Mcdonald, R. I., … Griscom, B. W. (2018). Natural climate solutions for the United States. *Science Advances, 4*(11). https://doi.org/10.1126/sciadv.aat1869

Finney, D. M., Buyer, J. S., & Kaye, J. P. (2017). Living cover crops have immediate impacts on soil microbial community structure and function. *Journal of Soil and Water Conservation, 72*(4), 361–373. https://doi.org/10.2489/jswc.72.4.361

Finney, D. M., & Kaye, J. P. (2016). Functional diversity in cover crop polycultures increases multifunctionality of an agricultural system. *Journal of Applied Ecology*, *54*(2), 509-517. https://doi.org/10.1111/1365-2664.12765

Finney, D. M., White, C. M., & Kaye, J. P. (2016). Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. *Agronomy Journal, 108*(1), 39-52. https://doi.org/10.2134/agronj15.0182

Gliessman, S. R. (2006). *Agroecology: the ecology of sustainable food systems*. CRC press.

Hansen, N. C., Allen, B. L., Anapalli, S., Blackshaw, R. E., Lyon, D. J., & Machado, S. (2016). Dryland Agriculture in North America. In *Innovations in dryland agriculture* (pp. 415-441). Springer.

Haramoto, E. R., & Gallandt, E. R. (2004). Brassica cover cropping for weed management: A review. *Renewable agriculture and food systems*, *19*(4), 187-198. https://doi.org/10.1079/RAFS200490

Hartwig, N. L., & Ammon, H. U. (2002). Cover crops and living mulches. *Weed Science*, *50*(6), 688-699. https://doi.org/10.1614/0043-1745(2002)050[0688:AIACCA]2.0.CO;2

Kaspar, T. C., Jaynes, D. B., Parkin, T. B., Moorman, T. B., & Singer, J. W. (2012). Effectiveness of oat and rye cover crops in reducing nitrate losses in drainage water. *Agricultural Water Management, 110*, 25-33. https://doi.org/10.1016/j.agwat.2012.03.010

Kaspar, T. C., & Singer, J. W. (2011). *The use of cover crops to manage soil.* In Soil management: Building a stable base for agriculture, (pp. 321-337). https://doi.org/10.2136/2011.soilmanagement.c21

Kaye, J. P., & Quemada, M. (2017). Using cover crops to mitigate and adapt to climate change. a review. *Agronomy for Sustainable Development*, *37*(1), 4. https://doi.org/10.1007/s13593-016-0410-x

Keesstra, S., Nunes, J., Novara, A., Finger, D., Avelar, D., Kalantari, Z., & Cerdà, A. (2018). The superior effect of nature based solutions in land management for enhancing ecosystem services. *Science of the Total Environment*, *610*, 997-1009. https://doi.org/10.1016/j.scitotenv.2017.08.077

Kell, W. & McKee, R. (1936). Farmer's Bulletin no. 1758: Cover Crops for Soil Conservation. United States Department of Agriculture, Washington, DC.

Kim, N., Zabaloy, M. C., Guan, K., & Villamil, M. B. (2020). Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biology and Biochemistry*, *142*, 107701. https://doi.org/10.1016/j.soilbio.2019.107701

Kladivko, E. J., Frankenberger, J. R., Jaynes, D. B., Meek, D. W., Jenkinson, B. J., & Fausey, N. R. (2004). Nitrate leaching to subsurface drains as affected by drain spacing and changes in crop production system. *Journal of Environmental Quality, 33*, 1803-1813. https://doi.org/10.2134/jeq2004.1803

Kramberger, B., Gselman, A., Janzekovic, M., Kaligaric, M., & Bracko, B. (2009). Effects of cover crops on soil mineral nitrogen and on the yield and nitrogen content of maize. *European Journal of Agronomy*, *31*(2), 103-109. https://doi.org/10.1016/j.eja.2009.05.006

Lehman, R. M., Acosta-Martinez, V., Buyer, J. S., Cambardella, C. A., Collins, H. P., Ducey, T. F., Halvorson, J. J., Jin, V. L., Johnson, J. M., Kremer, R.J., & Stott, D. E. (2015). Soil biology for resilient, healthy soil. *Journal of Soil and Water Conservation*, *70*(1), 12A-18A. https://doi.org/10.2489/jswc.70.1.12A

Lugato, E., Leip, A., & Jones, A. (2018). Mitigation potential of soil carbon management overestimated by neglecting N_2O emissions. *Nature Climate Change*, *8*(3), 219–223. https://doi.org/10.1038/s41558-018-0087-z

Martínez-García, L. B., Korthals, G., Brussaard, L., Jørgensen, H. B., & De Deyn, G. B. (2018). Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties. *Agriculture, Ecosystems & Environment*, *263*, 7-17. https://doi.org/10.1016/j.agee.2018.04.018

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*(3), 1–19. https://doi.org/10.1002/eap.2278

Millennium Ecosystem Assessment. (2005). Ecosystems and Human Well-Being: Synthesis. Island Press, Washington, DC.

Miguez, F. E., Archontoulis, S, & Basche, A. D. (2016). *Predicting Long-Term Cover Crop Impacts on Soil Quality Using a Cropping Systems Model (2016 Project Summaries)*. Leopold Center for Sustainable Agriculture, Iowa State University. Ames, IA. http://lib.dr.iastate.edu/leopold_grantreports/504

Muhammad, I., Wang, J., Sainju, U. M., Zhang, S., Zhao, F., & Khan, A. (2021). Cover cropping enhances soil microbial biomass and affects microbial community structure: A meta-analysis. *Geoderma*, *381*, 114696. https://doi.org/10.1016/j.geoderma.2020.114696

National Research Council. (1993). *Soil and water quality: An agenda for agriculture*. National Academies Press.

Natural Resources Conservation Service. (2021). *Unlock the Secrets in the Soil: Principles for High Functioning Soil.* United States Department of Agriculture. https://www.nrcs.usda.gov/sites/default/files/2022-10/SOILS%20Principles%20of%20High%20Functioning_Factsheet%202021.pdf

Natural Resources Conservation Service. (2022). *A brief history of NRCS*. United States Department of Agriculture. https://www.nrcs.usda.gov/about/history/brief-history-nrcs

Nevins, C. J., Nakatsu, C., & Armstrong, S. (2018). Characterization of microbial community response to cover crop residue decomposition. *Soil Biology and Biochemistry*, *127*, 39-49. https://doi.org/10.1016/j.soilbio.2018.09.015

Olson, K., Ebelhar, S. A., & Lang, J. M. (2014). Long-term effects of cover crops on crop yields, soil organic carbon stocks and sequestration. *Open Journal of Soil Science*, *4*(8). http://doi.org/10.4236/ojss.2014.48030

Peterson, G. A., & Westfall, D. G. (2019). Management of dryland agroecosystems in the central Great Plains of Colorado. In *Soil organic matter in temperate agroecosystems* (pp. 371-380). CRC Press.

Quemada, M., Lassaletta, L., Leip, A., Jones, A., & Lugato, E. (2020). Integrated management for sustainable cropping systems: Looking beyond the greenhouse balance at the field scale. *Global Change Biology*, *26*(4), 2584–2598. https://doi.org/10.1111/gcb.14989

Robertson, G. P., & Swinton, S. M. (2005). Reconciling agricultural productivity and environmental integrity: a grand challenge for agriculture. *Frontiers in Ecology and the Environment*, *3*(1), 38-46. https://doi.org/10.1890/1540-9295(2005)003[0038:RAPAEI]2.0.CO;2

Roe, S., Streck, C., Beach, R., Busch, J., Chapman, M., Daioglou, V., Deppermann, A., Doelman, J., Emmet-Booth, J., Engelmann, J., Fricko, O., Frischmann, C., Funk, J., Grassi, G., Griscom, B., Havlik, P., Hanssen, S., Humpenöder, F., Landholm, D., … Lawrence, D. (2021). Land-based measures to mitigate climate change: Potential and feasibility by country. *Global Change Biology, 27,* 6025-6058. https://doi.org/10.1111/gcb.15873

Ruis, S. J., Blanco-Canqui, H., Creech, C. F., Koehler-Cole, K., Elmore, R. W., & Francis, C. A. (2019). Cover crop biomass production in temperate agroecozones. *Agronomy Journal*, *111*(4), 1535-1551. https://doi.org/10.2134/agronj2018.08.0535

Schipanski, M. E., Barbercheck, M., Douglas, M. R., Finney, D. M., Haider, K., Kaye, J. P., Kemanian, A. R., Mortensen, D. A., Ryan, M. R., Tooker, J., & White, C. (2014). A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agricultural Systems*, *125*, 12–22. https://doi.org/10.1016/j.agsy.2013.11.004

Snapp, S. S., Swinton, S. M., Labarta, R., Mutch, D., Black, J. R., Leep, R., ... & O'neil, K. (2005). Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agronomy Journal*, *97*(1), 322-332. https://doi.org/10.2134/agronj2005.0322a

Sperow, M., Eve, M., & Paustian, K. (2003). Potential soil C sequestration on US agricultural soils. *Climatic Change*, *57*(3), 319-339. https://doi.org/10.1023/A:1022888832630

Stark, C. H., Condron, L. M., O'Callaghan, M., Stewart, A., & Di, H. J. (2008). Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralisation resulting from farm management history and organic matter amendments. *Soil Biology and Biochemistry*, *40*(6), 1352-1363. https://doi.org/10.1016/j.soilbio.2007.09.025

Tilman, D. (1999). Global environmental impacts of agricultural expansion: the need for sustainable and efficient practices. *Proceedings of the National Academy of Sciences*, *96*(11), 5995-6000. https://doi.org/10.1073/pnas.96.11.5995

Tilman, D., Fargione, J., Wolff, B., D'antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W. H., Simberloff, D., & Swackhamer, D. (2001). Forecasting agriculturally driven global environmental change. *Science*, *292*(5515), 281-284. https://doi.org/10.1126/science.1057544

Tonitto, C., David, M. B., & Drinkwater, L. E. (2006). Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: A meta-analysis of crop yield and N dynamics. *Agriculture, Ecosystems & Environment*, *112*(1), 58-72. https://doi.org/10.1016/j.agee.2005.07.003

United States of America. (2021). *The Long-Term Strategy of the United States: Pathways to Net-Zero Greenhouse Gas Emissions by 2050.* https://www.whitehouse.gov/wp-content/uploads/2021/10/US-Long-Term-Strategy.pdf

U.S. Department of Agriculture. (2022). *Cover Crops Play a Starring Role in Climate Change Mitigation.* Farm Service Agency, Kansas State Office. https://www.fsa.usda.gov/Assets/USDA-FSA-Public/usdafiles/State-Offices/Kansas/newsletters/2022%20September%20Newsletter.pdf

Wang, J., Zhang, S., Sainju, U. M., Ghimire, R., & Zhao, F. (2021). A meta-analysis on cover crop impact on soil water storage, succeeding crop yield, and water-use efficiency. *Agricultural Water Management*, *256*, 107085. https://doi.org/10.1016/j.agwat.2021.107085

Wick, A., Berti, M., Lawley, Y., & Liebig, M. (2017). Integration of annual and perennial cover crops for improving soil health. In *Soil health and intensification of agroecosystems* (pp. 127-150). Academic Press.

CHAPTER 2: CLIMATE MITIGATION POTENTIAL OF COVER CROPS IN THE U.S.

2.1 Introduction

Increasingly ambitious reductions in greenhouse gas (GHG) emissions will be required to achieve less than 2°C warming target set by the Paris Agreement (Rockström et al., 2017). The most recent U.S. GHG emissions targets reflect this urgency, with a goal of 50% reduction by 2030 and a net-zero economy-wide emissions by 2050 (United States of America, 2021). These goals must be achieved through aggressive reductions in fossil fuel combustion, but natural climate solutions (NCS) will also be necessary to adequately accelerate annual GHG emissions reduction and to offset emissions in sectors that cannot fully decarbonize in the short term (Griscom et al., 2018; Bossio et al., 2020; Bistline et al., 2022). Proposed scenarios to meet emissions targets involve increasing the U.S. land C sink by 250 Mt CO_2e year^{-1} through land-based NCS, and an estimated 35% of this mitigation potential is attributed to improved management of agricultural lands (United States of America, 2021; Roe et al., 2021).

Cover crops (CC) have received considerable attention and investment as a cost-effective NCS (Fargione et al., 2018). Grown in between cash crop seasons during periods which would otherwise be fallow, CC increase overall C inputs to the soil and control erosion, resulting in rates of C sequestration that are relatively high compared to other management practices (Kaye & Quemada, 2017; Fargione et al., 2018). Cover crops also have numerous on-farm co-benefits, and they are widely promoted to improve soil health and sustain cropping system productivity (Daryanto et al., 2018). To leverage the mitigation potential of CC and other agricultural practices to meet updated emissions goals, the U.S. Department of Agriculture (USDA) recently launched the Climate Smart Commodities program, a 2.8 billion USD initiative that aims to sequester 50 Mt CO_2e over a five-year period (USDA, 2022).

The estimated net GHG mitigation potential of CC in the U.S. varies widely. Estimates range from 64 to 103 Mt CO_2e year^{-1} (Sperow et al., 2003; Fargione et al., 2018; Roe et al., 2021). Area-based C sequestration rates attributed to CC are also highly variable, ranging from 1.17 to 3.78 t CO_2e ha^{-1} year^{-1} in recent meta-analyses, which depend on site-specific factors such as soil texture, tillage regime, CC frequency, and CC biomass production (Abdalla et al., 2019; Vicente-Vicente et al., 2016; Poeplau & Don, 2015; McClelland et al., 2021a). Estimates of GHG mitigation potential are sensitive to the geographic distribution of CC adoption because of the regional heterogeneity in environmental conditions and farm management practices; accounting for this variability would both reduce the uncertainty of previous estimates and inform where to prioritize CC incentives to maximize public GHG benefits.

The feasibility of CC adoption will also influence GHG mitigation potential. Current CC adoption rates are 5.1% nationally despite well-documented co-benefits for farm management (Wallander et al., 2021). Low adoption rates are partially attributed to farmer preferences and economic concerns, such as risk aversion, management complexity, and variable impacts on net profit (Plastina et al., 2020; Arbuckle & Roesch-McNally, 2015); however, research shows that these can be overcome to a large extent with incentive-based programs and technical assistance (Piñeiro et al., 2020). At the same time, low adoption is also partially due to logistical barriers that would require drastic changes in management. A study of ten midwestern counties found that 19 to 66% of corn and soy farms were not compatible with winter CC due to winter grain production or late cash crop harvest where tillage prevented CC interseeding (Kladivko et al., 2014). Such findings suggest that assessments of GHG mitigation potential should consider current management and crop rotations to exclude ineligible cropland to provide more realistic estimates.

While assessments of CC mitigation potential have primarily focused on C sequestration, recent studies highlight the important role of N_2O emissions in evaluating GHG benefits (IPCC, 2014; Guenet et al., 2020; Lal, 2016). Nitrous oxide is a potent GHG which can, in some cases, completely negate the GHG benefits achieved from C sequestration, particularly on longer time scales when C sequestration benefits are reduced (Lugato et al., 2018; Quemada et al., 2020). Nitrogen additions from legume CC and higher labile soil organic matter can enhance N_2O emissions whereby the cropping system can go from a net sink to a net source of GHG (Booth et al., 2005; Lugato et al., 2018). Conversely, GHG benefits can be enhanced by CC with high C:N ratios which can reduce inorganic N pools and rates of denitrification (Muhammad et al., 2019; Basche et al., 2016). Many management and site conditions affect the magnitude and direction of N_2O impacts, including CC C:N ratio, precipitation patterns, N fertilizer adjustments, and CC termination methods (Han et al., 2017; Basche et al., 2014). These factors and the dynamic nature of processes influencing N_2O emissions contribute to large variability of N_2O impacts across sites. For example, a meta-analysis by Basche et al. (2014) found that 40% of included studies demonstrated decreased N_2O emissions due to CC while 60% increased N_2O emissions. Understanding CC impacts on N_2O is therefore critical for quantifying overall potential mitigation benefits.

With increased investment and the important role of CC in national GHG emission reduction plans, updated and region-specific estimates of CC GHG mitigation potential are needed. We aimed to quantify the GHG mitigation potential of widespread CC adoption, including both additional sequestration of soil organic carbon (SOC) and change in direct N_2O emissions due to potential CC adoption across the continental U.S. We used GHG emissions inventory data to reflect the most accurate and current agricultural management practices and a process-based model to account for variability of GHG mitigation rates across time and space. We also estimated the potential for increased N_2O emissions due to CC adoption to offset C sequestration benefits, and identified how climate, soil and management affects this relationship and overall GHG mitigation potential.

2.2 Methods

2.2.1 The DayCent Model

The study was conducted using the DayCent model, a terrestrial ecosystem model which simulates C and N fluxes among the atmosphere, vegetation, and soil using a daily time-step and to a depth of 30 cm in the soil profile (Del Grosso et al., 2001, Parton et al., 1998). The model partitions SOC into pools with different potential decay rates (active, slow and passive), and SOC pool size is influenced by both quantity and quality of above- and belowground plant inputs (Parton et al., 1988). The N submodel is affected by decomposition rates of SOM pools and also accounts for fertilizer applications. The model was calibrated with Bayesian methods and observations from long-term experiments and is widely used in agroecosystems (Gurung et al., 2020). Comparisons with field data show that simulated net primary production, SOC stocks, and N_2O fluxes agree well with observed data at both the site and regional scales (Del Grosso et al., 2005).

DayCent is well-suited for projections of CC adoption since it considers characteristics that may vary among CC species, including biomass quality and quantity, growth periods, and potential N fixation. Our modeling approach takes advantage of recent work by McClelland et al. (2021b) that improved DayCent's ability to predict CC biomass quality and quantity with parameterization for a range of CC species, including cereal rye, clover, sunn hemp, and vetch.

2.2.2 Point selection and model inputs

Simulations were based on the National Resources Inventory (NRI) survey, which was initiated in 1979 by the Natural Resources Conservation Service (NRCS) to collect information on soil properties and land management annually throughout the U.S. (USDA-NRCS, 2016). The survey includes a stratified two-stage random sample design encompassing 349,464 agricultural locations (USDA-NRCS, 2016). Each location has an expansion factor that is used to estimate the total land area that the survey location represents in the total population of agricultural fields across the United States. We excluded NRI locations with production of sugarcane and less-common horticultural crops. We also excluded survey locations which did not have a winter fallow period that could be planted with winter CC (i.e., grasslands, perennial cropping systems and continuous winter grain systems). The remaining 132,319 NRI locations included in our study represent rotations with alfalfa hay, barley, corn, cotton, grass hay, grass-clover hay, oats, peanuts, potatoes, rice, sorghum, soybeans, sugar beets, sunflowers, tobacco, and wheat production, which constitutes approximately 94.1 Mha, or 70% of total national cropland (US-EPA, 2022).

Model inputs included daily weather data, soil data, and detailed management information, such as plant type, cultivation and planting schedules, and amount and timing of inputs. Daily weather data from 1979 to 2015, including daily maximum and minimum temperatures and precipitation, were sourced from the 4-kilometer gridded PRISM climate dataset (PRISM Climate Group, 2018) and the Soil Survey Geographic Database (SSURGO) was used to characterize soil properties, including soil texture, depth and pH, at each survey location (Soil Survey Staff, 2019).

Cover crop adoption rates were imputed for NRI survey locations using a hot deck method (Andridge & Little, 2010). Winter CC adoption was assumed to be negligible before 1990 and increase at a linear rate until reaching regional adoption rates captured in the 2000-2005 CEAP survey data (USDA-NRCS, 2018). After 2005, winter CC are randomly assigned or removed to match adoption rates reported in the 2012 and 2017 USDA Census of Agriculture (USDA-NASS, 2012; USDA-NASS, 2017).

While crop histories were sourced from NRI survey data, organic and synthetic fertilization, tillage practices, irrigation management, and CC management were imputed for each NRI survey location using statistical methods employed in the U.S. National GHG Inventory (US-EPA, 2022). Nitrogen management imputations were based on data from the NRCS Conservation Effects and Assessment Project (CEAP) and other USDA surveys (USDA-ERS, 1997; Brakebill & Gronberg, 2017; USDA-NASS, 2018; USDA-NRCS, 2018). Tillage management was based on data from the Conservation Technology Information Center (CTIC) for 1980 through 2004 (CTIC, 2004), CEAP survey for 2000 through 2005 (USDA-NRCS, 2018), and USDA Agricultural Resource Management surveys for 2002 through 2015 (Claasen et al., 2018). Six imputed management scenarios were used for model simulations. Detailed methods for the statistical imputation methods can be found in US-EPA (2022).

2.2.3 Cover crop adoption scenarios and model simulation

Two CC adoption scenarios were simulated, including a baseline adoption scenario and a high adoption scenario. In the baseline scenario, CC were only simulated at NRI locations which implemented CC between 2010 and 2015 according to imputation methods detailed above. Since our baseline adoption scenario reflects 2015 CC adoption, baseline adoption rates vary by CEAP region (Table S2.1). Points including CC in the baseline were also cover cropped in the high adoption scenario, and remaining points were randomly selected for CC adoption until a target 80% adoption rate was achieved in each CEAP region. As the selected points could include mixed cash crop systems, CC were not simulated in years which had a winter grain as the subsequent crop. This caused total area planted to CC in any given year (54.7 Mha) to be less than total area with CC incorporated throughout the course of the study (74 Mha).

We note that the baseline scenario assumes no new CC adoption throughout the study period, which is likely an underestimate of true changes in adoption. The 80% adoption was chosen for the high adoption scenario since it is significantly higher than current adoption rates (5.1%; Wallander et al., 2021) and full adoption is highly unlikely as not all barriers to adoption can be overcome with policy (Plastina et al., 2020; Piñeiro et al., 2020).

2.2.4 Historical simulations

To achieve initial values for state variables in the model (i.e., SOC, mineral N), DayCent simulations prior to 2015 followed methodology and input data outlined in the U.S. National Greenhouse Gas Inventory (US-EPA, 2022). First, steady-state organic matter stocks were achieved by simulating native ecosystems for several thousand years until the approximate date of conversion to cropland. This date, as well as crop history prior to 1979 was determined by the Major Land Resource Region (MLRA) level for each survey location (USDA-NRCS, 1997; Williams & Paustian, 2005). Generally, simulations were conducted with low input agricultural practices until 1950, and then fertilizer rates and more productive cropping varieties were introduced into the simulations during the latter part of the 20th century. (More information about the historical simulations is provided in US-EPA, 2022.)

Management practices and crop rotations from 2010 to 2015 for each of the six imputed management scenarios were repeated in 6-year cycles. Each NRI survey location was therefore simulated 12 times for each combination of management imputation and adoption scenario. Weather data from 2008-2015 was repeated in 8-year cycles. The starting year of the simulation was set at 2015 and included an initial spin-up period until 2019. Adoption scenarios were implemented in the year 2020 until 2039. This time frame was adopted to remove bias for incomplete cycles of input data (weather and management) and to be consistent with the GHG methods guidance provided by the Intergovernmental Panel on Climate Change (Ogle et al., 2006).

2.2.5 Cover crop type and management

Cover crop selection and planting and termination dates varied among Conservation Effects Assessment Project (CEAP) regions, which are agricultural ecoregion classifications developed by the U.S. Department of Agriculture (USDA-NRCS, 2018). Planting and termination dates were informed by Conservation Technology Information Center (CTIC) survey data and guidelines from the Midwest Cover Crops Council (MCCC) and Sustainable Agriculture Research and Education (MCCC, 2020; Schonbeck & Morse, 2006; CTIC, 2017). Simulated CC types included crimson clover (*Trifolium incarnatum* L.), cereal rye (*Secale cereale* L.), and radish (*Raphanus sativus*) to reflect most common CC species in the CTIC data (CTIC, 2017). Clover was simulated if the cash crop was harvested 6 to 8 weeks before the average frost date for the region and the next crop in the rotation was not a legume (Table S2.2). If the next crop was a legume, cereal rye was simulated, except in major wheat-producing regions (Northern Great Plains, Southern Great Plains, North Central, and East Central regions), where radish was simulated instead. Cereal rye was planted if the cash crop was harvested within 6 weeks of the average frost date for the region.

Cover crop planting dates were simulated to be within one week after cash crop harvest and terminated with herbicides. Over-wintering CC (clover and cereal rye) were terminated 10 to 14 days before planting cash crops, and winter-kill CC (radish) were terminated in January. Fertilization rates were not adjusted for N supplied by legumes in our study, which could be further investigated in future model-based assessments.

2.2.6 Estimating national mitigation potential and associated uncertainty

A Monte Carlo approach adapted from Ogle et al. (2010) was used to quantify uncertainty associated with management input data and error in model parameters. Six imputations of management history for the NRI survey locations were used to represent uncertainty surrounding management practices, such as mineral fertilization rates, manure amendments and tillage practices. Uncertainty in model parameters and structure was estimated using an empirical approach in which linear mixed effect models were derived by comparing modelled soil C stocks and N_2O fluxes from DayCent with observed data from long term experiments (Ogle et al., 2007; Del Grosso et al., 2010). The 'true' SOC stocks and N_2O fluxes, as represented by the measurement data, are a function of the DayCent predictions and, in the case of N_2O, additional covariates which include crop type and freeze-thaw cycling were added to address bias in DayCent model predictions. Random effects associated with region, site, and year were also included in these models.

The Monte Carlo analysis included 1000 iterations for each CC scenario. Each iteration included a random selection of one of the six management imputations and a random selection of fixed and random parameter values for the empirical linear mixed effects model (Ogle et al., 2010). For each iteration, SOC stocks and N_2O emissions were determined for each NRI location for each year from 2020 to 2039. NRI expansion factors associated with each point were then used to scale outcomes to represent total eligible cropland included in the study, aggregated by CEAP region and at the national scale. The GHG mitigation potential of CC was quantified for each Monte Carlo iteration as the decrease in net GHG flux between the high and baseline CC adoption scenarios due to C sequestration (dSOC), N2O emissions (dN_2O) and net GHG flux (calculated by adding dSOC and dN_2O converted to CO_2-equivalent). Positive dSOC values therefore indicate additional

C sequestration due to CC, positive dN_2O values indicate increases in N_2O flux due to CC, and positive GHG mitigation potential indicates decreases in net GHG flux due to CC. Estimates are based on the mean and standard deviation among all Monte Carlo iterations.

2.2.7 Analysis for site-level controls on GHG mitigation potential

We also explored the influence of management, climate, and soil factors on C sequestration rates and change in N_2O emissions due to CC adoption. For each survey location, we averaged the results from the Monte Carlo simulation (see previous section) to estimate the annual C sequestration rate and N_2O emissions for the baseline and CC scenario. Differences in average annual dSOC and dN_2O rates with and without CC were calculated for each survey location. These differences were aggregated at the county level, and the means and standard deviations were also calculated for each CEAP region. Net GHG balance due to CC adoption was also calculated for each location and the percent per region with positive balances was determined.

The dSOC and dN_2O rates were statistically analyzed based on variation in soil, climate, and management factors for each site which have previously been shown to be influential in GHG mitigation due to CC, including sand content, tillage regime, annual N fertilizer rates, mean annual precipitation (MAP), potential evapotranspiration (PET), frequency of legume CC, and average annual CC biomass C (McClelland et al., 2021a; Basche et al., 2014). Average annual CC biomass C reflected both biomass production and the number of years that CC were incorporated into the rotation. Tillage regime was included as a continuous variable to account for changes among years and among management imputations. All years with full tillage were assigned a value of 2, reduced tillage was assigned 1 and no-till was assigned 0 and the average of all years was included in the regression. Multiple linear mixed-effect regression was performed to analyze changes in C sequestration rates, including all individual site factors and an interaction between proportion of legume CC and average CC biomass as fixed effects. The CEAP region was included as a random effect.

Multiple linear mixed-effect regression was also performed to explore the relationship between dN_2O and dSOC rates. All site factors and the interaction between proportion of legume CC and average CC biomass were included as fixed effects as well as dSOC rate and its interaction with site factors. CEAP region was included as a random effect. In all regression analysis, dSOC and dN_2O rates were log transformed after adding a constant to each value to avoid issues with negative values. The lme4 package in R statistical software was used for regression analysis (Bates et al., 2015; R Core Team, 2021).

Due to large sample size, p-values and Akaike Information Criterion were not appropriate metrics to evaluate the regressions. Instead, inclusive R^2 and standardized beta coefficients were calculated using the partR2 package in R (Stoffel et al., 2020). Factors which had inclusive R^2 greater than 10% were highlighted as having substantial explanatory power on dSOC and dN_2O. To visualize the interaction between proportion of legume and dSOC, CC type was defined categorically for each location as either legume (>90% legume CC), mixed (10%-90% mixed CC), and non-legume (<10% legume CC).

2.3 Results

2.3.1 National GHG mitigation potential

Adopting CC on an additional 74 Mha of cropland throughout the U.S. (83% of total eligible area) has the potential to sequester an additional 39.0 ± 24.3 Mt CO_2e per year nationally through 2039 in the top 30 cm of soil (Fig. 2.1; Table S2.3). This translates to a 38% reduction in the net GHG balance of the cropland included in the study, as the net GHG flux under the baseline scenario was an estimated 101.4 ± 16.9 Mt CO_2e $year^{-1}$. The majority of this GHG mitigation (36.1

± 7.9 Mt CO_2e year^{-1}) is attributed to additional C sequestration, which declined over time, starting at 44.5 ± 14.4 Mt CO_2e year^{-1} and reaching the lowest level of 25.1 ± 12.2 Mt CO_2e year^{-1} in the last two years of the study period (Fig. 2.2). Nitrous oxide emissions were reduced only slightly with increased CC adoption, estimated at -2.85 ± 22.8 Mt CO_2e year$^{=1}$, and introduced substantial variability to overall net GHG balance estimates (Fig. 2.1).

The GHG mitigation potential due to CC adoption was unevenly distributed among CEAP regions and was proportional to the area of cropland eligible in each region (Fig. 2.1). Of the total eligible area (94.1 Mha), 79% is concentrated in mid-continental CEAP regions, including the Northern Plains, Southern Plains, and North Central (Fig. 2.1; Table S2.1). As a result, these regions contribute to the majority (71%) of GHG mitigation potential (Fig. 2.1; Table S2.3). Western CEAP regions (Northwest Coastal, California Coastal, Northwest Non-Coastal, and Southwest Non-Coastal) had only 2% of total cropland eligible for CC and therefore contributed relatively little to total GHG mitigation. Eastern regions, such as Lower Mississippi, Southeast, and East Central had more total eligible cropland but higher baseline adoption rates, so potential for increased adoption was lower (Fig. 2.1). These regions made up 23% of total mitigation potential.

2.3.2 Influence of management, climate, and soil factors on GHG mitigation rates

Change in C sequestration rates among newly adopted CC locations (dSOC) varied regionally (Fig. 2.3; Table 2.1). Multiple linear regression indicated that regional changes averaged from 0.33 ± 0.24 to 0.99 ± 0.39 t CO_2e ha^{-1} year^{-1} and were primarily driven by annual CC biomass C, a metric of both CC frequency and average CC biomass C at termination, which explained 62% of variability in dSOC rate (Table 2.2). Mean annual precipitation and PET also had high explanatory power (inclusive R^2= 27% and 13%, respectively). Regions with lower MAP such as the Western Non-Coastal regions and the Great Plains tended to have lower CC biomass production and dSOC rates that were less than the national average (Fig. 2.4; Table S2.4). Conversely, the Lower Mississippi and South Central regions were associated with higher precipitation, higher annual biomass production and dSOC (Fig. 2.4; Table S2.4). Locations in the California Coastal region were the exception to this pattern, where precipitation is low but there was high biomass production and higher rates of dSOC. This is likely because this region relies on irrigation to meet water demands rather than precipitation, as rice was the predominant cropping system among eligible CC locations in the California Coastal region (Table S2.2).

Change in N_2O emissions (dN_2O) was slight and highly variable with average regional changes between -0.05 ± 0.11 t CO_2e ha^{-1} $year^{-1}$ and 0.23 ± 0.22 t CO_2e ha^{-1} $year^{-1}$ (Table 2.1). Generally, higher C sequestration due to CC adoption was not associated with a trade-off of increased in N_2O emissions. The dSOC had low explanatory power for dN_2O (inclusive $R^2 = 0.2\%$) and the associated beta coefficient was less than 0 (Beta=-0.244; Table 2.2). Overall, 29.8% of locations had increased N_2O emissions with CC adoption, but annual net GHG flux still decreased in 97.9% of NRI survey locations with new CC adoption (Table 2.1).

Site factors included in regression analysis explained only 30% of variation in dN_2O (Table 2.2). An interaction between CC type and dSOC explained 14% of variability and further analysis showed that increased CC biomass only led to increased N_2O emissions in systems in which >90% of CC planted were legumes (Fig. 2.5; Table 2.2). This interaction also explains increased N_2O emissions with CC adoption in the California Coastal regions, where legume CC were almost always simulated due to CC selection rules and prevalent regional crop rotations (Table S2.2).

2.4 Discussion

2.4.1 National mitigation potential

Widespread adoption of CC throughout the U.S. has a GHG mitigation potential of 39.0 ± 24.1 Mt CO_2e year^{-1} nationally through the year 2039. This mitigation potential would offset total N_2O emissions from U.S. agriculture by 12% (US-EPA, 2022) and contribute to approximately 15% of the 250 Mt CO_2e increase in land C sink proposed to meet national GHG emission reduction targets (United States of America, 2021). Though this estimated potential could be part of a broader climate mitigation plan, it is substantially lower than previous estimates of CC mitigation potential which range from 65 to 103 Mt CO_2e year^{-1} (Table 2.3). These previous assessments represent an upper maximum, while the use of current land management data and the DayCent model revealed that mitigation potential due to CC adoption is limited by regional variability in net GHG flux, reduced C accrual over time following adoption, and uncertainty surrounding N_2O trade-offs.

The average dSOC rate on a per-area basis (0.49 ± 0.31 t CO_2e ha^{-1} year^{-1}) was less than averages published in meta-analyses and used in previous mitigation potential assessments involving CC (Table 2.3; Abdalla et al., 2019; Vicente-Vicente et al., 2016; Poeplau & Don, 2015; McClelland et al., 2021a). Published averages were more congruent with dSOC rates observed in regions with higher MAP and/or higher biomass production (i.e., Lower Mississippi, South Central, California Coastal), but change in SOC accrual decreased by more than half in drier regions such as North Central, Western Non-Coastal, and the Great Plains (Table 2.1; Fig. 2.3, 2.4). This is consistent with studies which show that SOC gains can be inconsistent and accrue more slowly in semi-arid regions, where C inputs are highly dependent on variable and low precipitation patterns (Blanco-Canqui et al., 2013; Simon et al., 2022). Despite making up 81% of eligible CC acres in the U.S. (Table S2.1), CC research is disproportionately more limited in these areas compared to wetter climates (Poeplau & Don, 2015), as empirical research is reflective of current adoption concentrated in the eastern U.S (Fig. 2.1). These regions are therefore largely underrepresented in meta-analyses, introducing bias in average C sequestration rates for large scale assessments that include a wet to dry climate gradient (Poeplau & Don, 2015; McClelland et al., 2021a; Abdalla et al., 2019; Vicente-Vicente et al., 2016).

Total C sequestration potential was also further reduced due to the consideration of winter grain production in our analysis. Winter wheat production makes up 13.9 Mha of U.S. cropland and is especially prevalent in the Great Plains regions where 32% of eligible CC area was located (Table S2.1, S2.2; USDA-NASS, 2022). As a result, CC were incorporated into crop rotations only 50-60% of study years on average across locations, and this frequency varied by region (Table S2.2). Cover crop frequency is shown to drive soil C accrual, both due to increased C inputs as well as increased microbial biomass and labile C pools (White et al., 2020; Brennan et al., 2017). Consequently, CC frequency was a key determinant of average annual CC biomass, which was the largest driver of C accrual rates across study locations (Table 2.2; Fig. 2.4).

There was a 44% reduction in C accrual rates over the course of the study period (Fig. 2.2). Simulated and field studies alike have shown that C sequestration rates slow as a new steady-state equilibrium is reached (Mazzoncini et al., 2011; Garcia-Gonzalez et al., 2018), with rates declining significantly after 20 years of CC adoption (Quemada et al., 2020; Lugato et al., 2018). Furthermore, Ogle et al. (*in prep*) showed that some of the potential for enhancing C sinks on U.S. cropland has already been realized through past adoption of CC and other recommended management practices (RMPs), such as conservation tillage, organic amendments, among others, which likely further reduced C sequestration rates. Enhancing the land C sink is not a long-term solution to offset GHG emissions, but rather could serve as a short-term sink to accelerate net GHG reductions before cumulative anthropogenic emissions cause irreversible effects on the climate system (Hansen et al., 2013; Lassaletta & Aguilera, 2015).

The use of other RMPs in conjunction with CC, or "stacking" conservation practices, could accelerate this net GHG sink potential (Lehman et al., 2019). Potential C sequestration rates on U.S. cropland are likely much higher than rates observed solely due to CC, as cropland set aside for conservation can sequester 2.1 t CO_2e ha^{-1} $year^{-1}$, though these rates also vary according to MAP (Post & Kwon, 2000; Piñeiro et al., 2009). For example, the mitigation potential of no-till itself has been challenged (Powlson et al., 2014), but associated improvements in soil structure can enhance C gains due to CC (Olson et al., 2014; Büchi et al, 2018). Increased water retention, reduced erosion, and improved soil structure under no-till can also increase CC biomass, shown in our study to be a key driver of C sequestration. No-till systems caused slightly greater rates of C sequestration in our analysis when combined with CC management (Table 2.2), though this effect was variable and may have been more pronounced if CC were not terminated with herbicides regardless of tillage regime (Ogle et al., *in prep*). Other RMPs such as biochar additions, organic amendments, and improved nutrient management can also enhance GHG benefits and can be used in combination with CC (Fargione et al., 2018; Bai et al., 2019).

2.4.2 Nitrous oxide trade-offs

Overall, N_2O trade-offs were primarily observed in systems with legume CC but did not appear to offset the climate benefits of C sequestration when aggregated across regions (Fig. 2.1, 2.5). These results are consistent with studies which show no change or an overall decrease in N_2O emissions with CC-associated C sequestration, particularly when N fertilizer rates are adjusted for N inputs from legume CC (Han et al., 2017; Quemada et al., 2020). Without proper adjustments, however, C sequestration benefits of legume CC can be completely offset by increased N_2O emissions (Quemada et al., 2020). These trade-offs can be particularly pronounced over time as C pools approach saturation levels (Fig. 2.2; Lugato et al., 2018); it is possible this phenomenon could have occurred in our study with the inclusion of more study years and a continuing decline in C sequestration rates. In these cases, trade-offs between GHG benefits and productivity can be alleviated by adjusting N fertilizer rates to reflect estimated N inputs from legume CC (Quemada et al., 2020; Han et al., 2017). Fertilizer savings can in turn contribute to greater economic feasibility of CC and reduce indirect GHG emissions associated with fertilizer production (Fargione et al., 2018).

Although simulated changes in N_2O emissions did not greatly impact the magnitude of total estimated GHG potential, the large uncertainty associated with N_2O impacts should be considered when using CC as a climate mitigation measure (Fig. 2.1). The level of uncertainty is common for modelled N_2O emissions, which are highly sensitive to management data and weather patterns (Del Grosso et al., 2010; Table S2.3). Nitrous oxide emissions are driven by multiple factors, including N availability, labile C, soil moisture, and soil physical properties, which are temporally and spatially dynamic, and emissions can increase or decrease depending on CC management choices (Basche et al., 2014). Improved understanding of these processes is needed to accurately predict net GHG impacts at the site level. Although national N_2O monitoring systems are likely to reduce uncertainty associated with estimating emissions in the future (Ogle et al., 2020), models such as DayCent have been shown to provide the most accurate estimation of N_2O fluxes currently available, which is critical when quantifying net GHG impacts for complex cropland management systems (dos Reis Martins et al, 2022).

2.4.3 Policy implications

The estimated mitigation potential in this study depends on significant increases in CC adoption, which will undoubtedly require substantial investments in incentive-based programs and technical assistance (Piñeiro et al., 2020; Kathage et al., 2022). Regional differences in GHG mitigation potential may inform where programs are prioritized, but trade-offs and other environmental objectives should be considered. For example, dSOC rates in the Lower Mississippi and the Southeast are greater than the national average (Table 2.1). These regions are also more likely to be associated with yield increases due to CC, meaning that incentive payments that must compensate for CC costs would be lower (Wang et al., 2021; Piñeiro et al., 2020). Conversely, the Great Plains area contributed 25% to the national mitigation potential, but CC are also shown to reduce yields in this region due to water limitations (Wang et al., 2021; Eash et al., 2021). In addition to increasing required incentive payments, reductions in productivity in the Great Plains could also increase land-use conversion to agriculture to maintain regional productivity levels, increasing indirect GHG emissions (leakage; Murray et al., 2007).

Our estimates of mitigation potential do not consider other ecosystem services which may be a stronger driver of CC adoption in some regions. In the Great Plains, CC adoption offers protection against erosion, a benefit which is critical for sustained crop productivity (Blanco-Canqui et al., 2015), and in the Mississippi River basin, nitrate retention with uptake by CC would address water quality concerns (Kladivko et al., 2014). More analysis is needed to examine where and to what extent public benefits outweigh incentive costs to identify feasible adoption rates. Incentive payments should also consider the potential lack of permanence and uncertainty associated with GHG mitigation benefits, particularly given the variable impacts of CC on N_2O emissions.

2.5 Conclusions

Natural climate solutions are a key component of the U.S. plan to achieve net-zero GHG emissions by 2050 (United States of America, 2021), but past assessments have presented upper potential maximum estimates. While CC are estimated to have a large mitigation potential compared to other agricultural NCS (Fargione et al., 2018), their C sequestration potential is 45-65% lower than previous U.S. estimates with the consideration of feasible CC frequency across the U.S., regional climate variation impacting the rates of C sequestration, and reduced C accrual over time. According to our analysis, C sequestration benefits are not offset by higher N_2O emissions unless adoption is exclusively legumes, but N_2O impacts introduce a large degree of uncertainty that should be considered in CC policy which aims to reduce GHG emissions. However, it may be possible to reduce the influence of legume CC on N_2O emissions if fertilization rates are also reduced for the next cash crop (Quemada et al., 2020).

Widespread adoption of CC can be part of a broader plan to accelerate GHG emissions reductions, while also providing important ecosystem services aside from climate regulation. Based on our estimates, CC alone could contribute 15% to the proposed increase in the U.S. land C sink (US-EPA, 2022; United States of America, 2021). The contribution from the agricultural sector could be further enhanced by increased innovation or the use of other RMPs in conjunction with CC. Our spatially-explicit analysis highlights regions where adoption of CC can have greater relative contributions to GHG mitigation; the Lower Mississippi region had highest mitigation rates on a per-area basis while the N Central region contributed most to total national mitigation potential. Additional regional analysis is recommended to balance total public ecosystem service benefits with necessary incentive payments and determine to what extent CC will contribute to climate regulation and the sustainability of the U.S. agricultural sector.

Table 2.1. Mean annual change in C sequestration (dSOC), annual N_2O emissions (dN_2O), and greenhouse gas mitigation from 2020 to 2039 for study locations with new cover crop adoption (n = 123,689). Standard deviation is presented in parentheses. The percent of study locations with favorable outcomes (dSOC > 0, dN_2O < 0 and GHG mitigation > 0) are also displayed nationally and by region.

Region	dSOC		dN_2O		GHG Mitigation		dSOC > 0	dN_2O < 0	GHG Mitigation > 0
	$t\ CO_2e\ ha^{-1}\ year^{-1}$		$t\ CO_2e\ ha^{-1}\ year^{-1}$		$t\ CO_2e\ ha^{-1}\ year^{-1}$		%	%	%
NW Coastal	0.532	(0.26)	0.030	(0.06)	0.502	(0.27)	100.0	33.3	100.0
CA Coastal	0.936	(0.52)	0.231	(0.22)	0.704	(0.41)	98.4	9.2	97.4
NW Non-Coastal	0.332	(0.24)	-0.005	(0.08)	0.337	(0.26)	94.3	51.1	92.4
SW Non-Coastal	0.356	(0.26)	0.027	(0.09)	0.328	(0.26)	94.7	34.2	91.9
N Great Plains	0.345	(0.23)	-0.034	(0.07)	0.379	(0.25)	95.4	67.5	95.7
S Great Plains	0.414	(0.22)	-0.052	(0.11)	0.466	(0.26)	98.3	62.6	98.0
N Central	0.431	(0.24)	-0.061	(0.07)	0.492	(0.26)	97.5	80.7	98.0
S Central	0.749	(0.36)	0.052	(0.14)	0.697	(0.40)	99.4	35.8	98.0
Lower MS	0.994	(0.39)	0.012	(0.14)	0.983	(0.41)	99.9	47.3	99.7
Northeast	0.499	(0.30)	-0.035	(0.05)	0.534	(0.32)	96.3	75.4	96.7
E Central	0.674	(0.28)	-0.023	(0.05)	0.697	(0.29)	99.1	69.5	99.0
Southeast	0.679	(0.27)	0.029	(0.05)	0.650	(0.28)	99.7	28.0	99.6
National	**0.492**	**(0.31)**	**-0.042**	**(0.09)**	**0.534**	**(0.32)**	**97.7**	**70.2**	**97.9**

Table 2.2. Multiple linear regression results for the influence of site-level soil, climate and management factors on change in mean annual C sequestration (dSOC) and N_2O emissions (dN_2O) due to newly adopted cover crops (CC). Outcomes were simulated over a 20-year study period (2020-2039). Beta represents the standardized coefficient and inclusive R^2 represents the variability explained by the site factor independent of other factors.

	dSOC		dN_2O	
Factor	Beta	Inclusive R^2	Beta	Inclusive R^2
dSOC	NA [1]	NA	-0.244	0.002
Sand Content	-0.104	0.040	0.253	0.142
Tillage [2]	-0.018	0.028	0.052	0.008
Average Annual N Fertilizer	-0.024	0.012	-0.065	0.000
MAP	0.141	0.2727	0.208	0.002
PET	-0.051	0.129	0.173	0.076
CC Legume Frequency	0.044	0.035	0.093	0.123
Annual CC Biomass C	0.798	0.6203	-0.433	0.020
CC Legume Frequency x Annual CC Biomass C	-0.026	0.2042	0.435	0.135
dSOC x Sand Content	NA	NA	0.079	0.133
dSOC x Tillage	NA	NA	0.090	0.007
dSOC x Annual CC Biomass C	NA	NA	0.314	0.018
dSOC x CC Legume Frequency	NA	NA	-0.071	0.111
Marginal R^2	0.646		0.301	

[1] NA = Not applicable. Factor not included in analysis. [2] Tillage was included as a continuous variable to account for changes among years and among management imputations. All years with full tillage were assigned a value of 2, reduced tillage was assigned 1 and no-till was assigned 0. Average tillage across years was included in the regression.

Table 2.3. Summary of previous assessments of the national mitigation potential of cover crops in the United States. Mitigation potential for the current study only considers additional C sequestration (dSOC) and excludes change in N_2O emissions for comparison with previous assessments.

Citation	Inclusion Criteria	Land Area	dSOC	Adoption Rate	Mitigation Potential
		Mha	t CO_2e ha^{-1} $year^{-1}$	%	Mt CO_2e $year^{-1}$
Sperow et al. (2003)	Exclude dry climate zones and crop rotations including winter grains	99	0.8	100	84
Fargione et al. (2018)	5 major annual crops (corn, soy, rice, wheat, cotton)	88	1.2	100	103
Roe et al. (2021)	Exclude dry climate zones and area with current cover crop adoption	103	0.7	90	65
Current study	All crop rotations with at least one year of winter fallow; excludes sugarcane and specialty vegetable crops with low national area	94	0.5	83	36

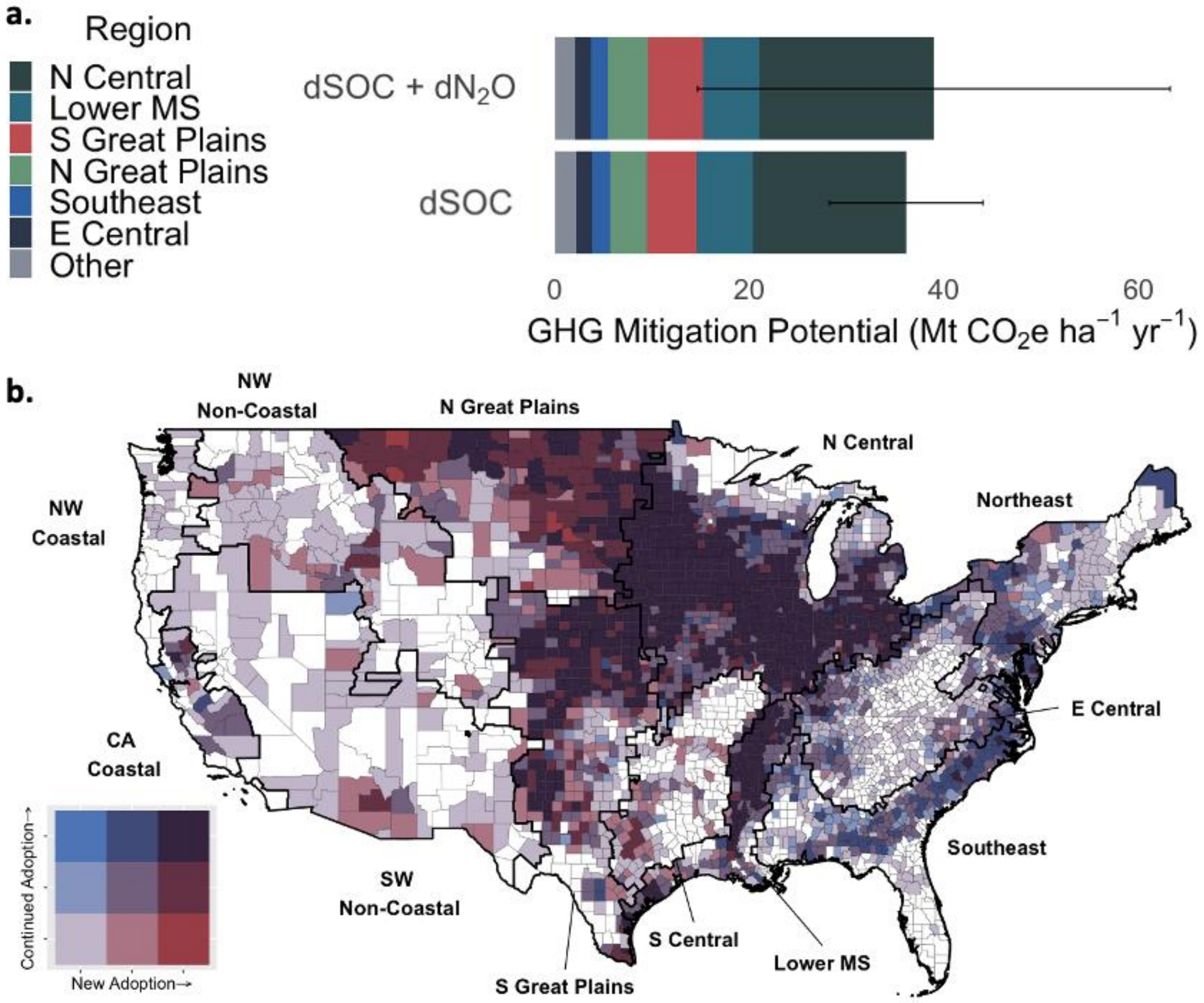

Figure 2.1. (a) Mean annual greenhouse gas (GHG) mitigation potential due to high cover crop adoption from 2020 to 2039 at the national scale and by region, considering both additional C sequestration (dSOC) and change in N$_2$O emissions (dN$_2$O). Error bars represent standard deviation of 1000 Monte Carlo iterations. (b) The national distribution of the total area of new (red) and continued (blue) CC adoption is also displayed, with data aggregated at the county level and regions delineated by thicker black lines. Color scales are separated by 33rd and 66th quantiles. Total area of new and continued adoption by region is displayed in Table S2.1.

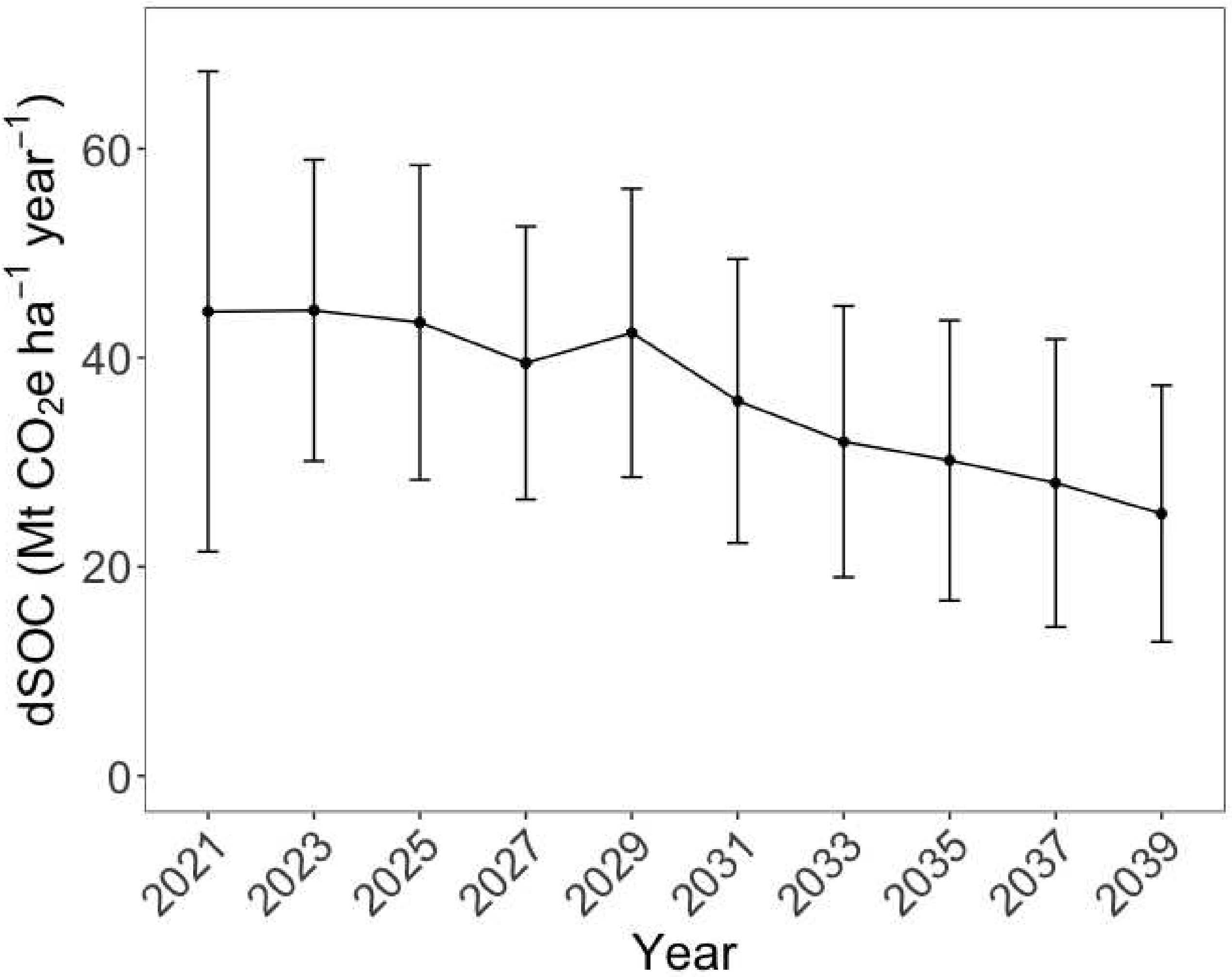

Figure 2.2. Mean annual change in C sequestration (dSOC) due to high national cover crop adoption. Annual dSOC averaged across 1000 Monte Carlo iterations is displayed for two-year intervals across the 20-year study period. Error bars represent the standard deviation of Monte Carlo iterations.

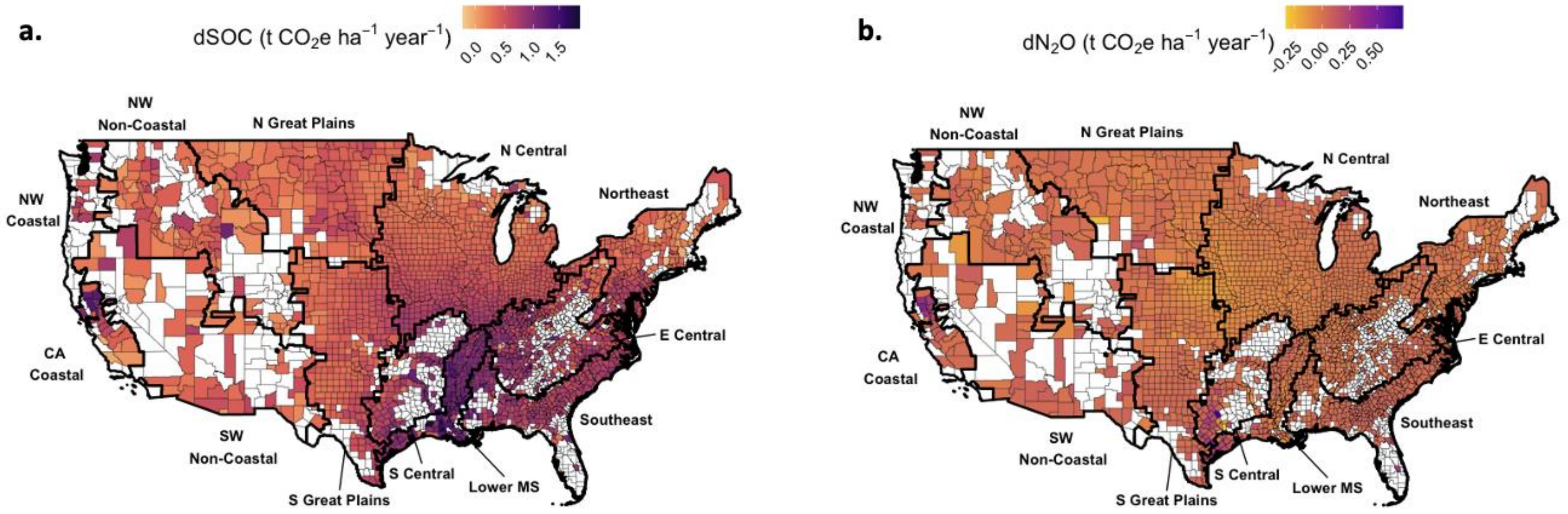

Figure 2.3. Mean (a) annual change in C sequestration (dSOC) and (b) annual N_2O emissions (dN_2O) from 2020 to 2039 for study locations with new cover crop adoption. Locations are aggregated at the county level. Thicker black lines delineate regions identified by the Conservation Effects Assessment Project (USDA-NRCS, 2018).

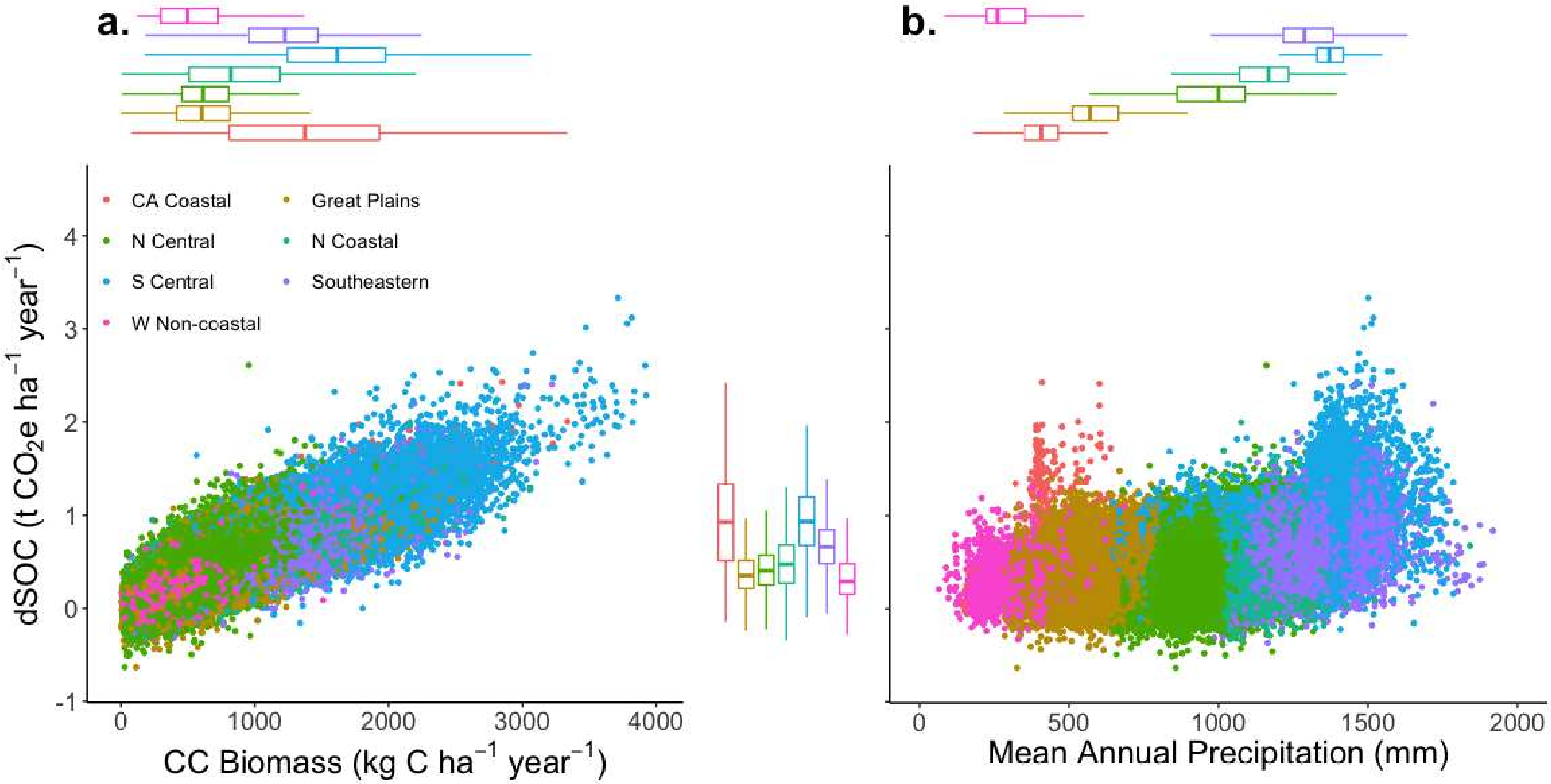

Figure 2.4. Relationship between (a) mean annual cover crop (CC) biomass C, (b) mean annual precipitation (MAP) and change in mean annual C sequestration rates (dSOC) for study locations with new cover crop adoption (n = 123,689). Points are color-coded by regions, which were identified by the Conservation Effects Assessment Project (USDA-NRCS, 2018) and condensed for clearer visualization. Great Plains = Northern and Southern Plains; W Non-Coastal = Northwest and Southwest Non-Coastal; S Central = Lower Mississippi and South Central; N Coastal = Northwest Coastal and Northeast. Boxplots which represent median and interquartile range of annual CC Biomass C, MAP, and dSOC are displayed in margins and aligned with appropriate axes.

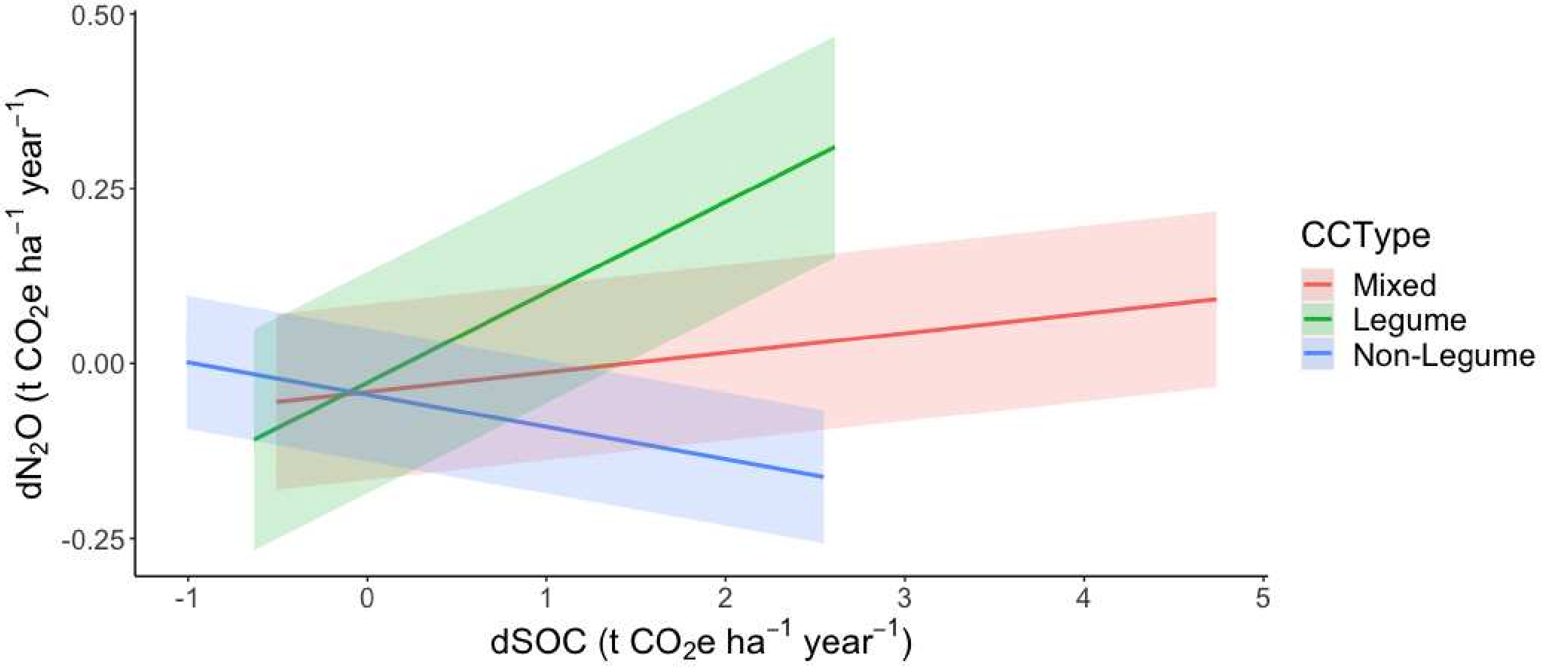

Figure 2.5. Relationship between mean annual change in C sequestration rates (dSOC) and N_2O emissions (dN_2O) for study locations with new cover crop (CC) adoption (n = 123,689). Outcomes were simulated over a 20-year study period (2020-2039). Regression lines represent simple linear regression of dSOC on dN_2O for each CC type; legume, mixed and non-legume CC indicates that legume CC were simulated > 90%, 10 - 90%, and < 10% of years in which CC were integrated, respectively. The shaded area represents the standard deviation.

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*(8), 2530-2543. https://doi.org/10.1111/gcb.14644

Andridge, R. R., & Little, R. J. (2010). A review of hot deck imputation for survey non-response. *International Statistical Review*, *78*(1), 40-64. https://doi.org/10.1111/j.1751-5823.2010.00103.x

Arbuckle, J. G., & Roesch-McNally, G. (2015). Cover crop adoption in Iowa: The role of perceived practice characteristics. *Journal of Soil and Water Conservation*, *70*(6), 418-429. https://doi.org/10.2489/jswc.70.6.418

Bai, X., Huang, Y., Ren, W., Coyne, M., Jacinthe, P. A., Tao, B., Hui, D., Yang, J., & Matocha, C. (2019). Responses of soil carbon sequestration to climate-smart agriculture practices: A meta-analysis. *Global Change Biology*, *25*(8), 2591-2606. https://doi.org/10.1111/gcb.14658

Basche, A. D., Archontoulis, S. V., Kaspar, T. C., Jaynes, D. B., Parkin, T. B., & Miguez, F. E. (2016). Simulating long-term impacts of cover crops and climate change on crop production and environmental outcomes in the Midwestern United States. *Agriculture, Ecosystems & Environment*, *218*, 95-106. https://doi.org/10.1016/j.agee.2015.11.011

Basche, A. D., Miguez, F. E., Kaspar, T. C., & Castellano, M. J. (2014). Do cover crops increase or decrease nitrous oxide emissions? a meta-analysis. *Journal of Soil and Water Conservation*, *69*(6), 471–482. https://doi.org/10.2489/jswc.69.6.471

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software, 67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Bistline, J., Abhyankar, N., Blanford, G., Clarke, L., Fakhry, R., McJeon, H., Reilly, J., Roney, C., Wilson, T., Yuan, M., & Zhao, A. (2022). Actions for reducing US emissions at least 50% by 2030. *Science*, *376*(6596), 922-924. https://doi.org/10.1126/science.abn0661

Blanco-Canqui, H., Holman, J. D., Schlegel, A. J., Tatarko, J., & Shaver, T. M. (2013). Replacing fallow with cover crops in a semiarid soil: Effects on soil properties. *Soil Science Society of America Journal*, *77*(3), 1026-1034. https://doi.org/10.2136/sssaj2013.01.0006.

Blanco-Canqui, H., Shaver, T. M., Lindquist, J. L., Shapiro, C. A., Elmore, R. W., Francis, C. A., & Hergert, G. W. (2015). Cover crops and ecosystem services: Insights from studies in temperate soils. *Agronomy journal*, *107*(6), 2449-2474. https://doi.org/10.2134/agronj15.0086

Booth, M. S., Stark, J. M., & Rastetter, E. (2005). Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. *Ecological monographs*, *75*(2), 139-157. https://doi.org/10.1890/04-0988

Bossio, D. A., Cook-Patton, S. C., Ellis, P. W., Fargione, J., Sanderman, J., Smith, P., Wood, S., Zomer, R. J., Von Unger, M., Emmer, I. M., & Griscom, B. W. (2020). The role of soil carbon in natural climate solutions. *Nature Sustainability*, *3*(5), 391-398. https://doi.org/10.1038/s41893-020-0491-z

Brakebill, J. W., & Gronberg, J. M. (2017). County-Level Estimates of Nitrogen and Phosphorus from Commercial Fertilizer for the Conterminous United States, 1987-2012: U.S. Geological Survey data release. https://doi.org/10.5066/F7H41PKX.

Brennan, E. B., & Acosta-Martinez, V. (2017). Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production. *Soil Biology and Biochemistry*, *109*, 188-204. https://doi.org/10.1016/j.soilbio.2017.01.014

Büchi, L., Wendling, M., Amossé, C., Necpalova, M., & Charles, R. (2018). Importance of cover crops in alleviating negative effects of reduced soil tillage and promoting soil fertility in a winter wheat cropping system. *Agriculture, Ecosystems & Environment*, *256*, 92-104. https://doi.org/10.1016/j.agee.2018.01.005

Claassen, R., Duquette, E. N., & Smith, D. J. (2018). Additionality in US agricultural conservation programs. *Land Economics*, *94*(1), 19-35. https://doi.org/10.3368/le.94.1.19

CTIC. (2004). *National crop residue management survey.* Conservation Technology Information Center. https://www.ctic.org/projects/Data_on_Conservation_Practices

CTIC. (2017). *Annual Report 2016-2017: Cover Crop Survey.* Conservation Technology Information Center. https://www.ctic.org/files/2017CTIC_CoverCropReport-FINAL.pdf

Daryanto, S., Fu, B., Wang, L., Jacinthe, P., & Zhao, W. (2018). Quantitative synthesis on the ecosystem services of cover crops. *Earth-Science Reviews*, *185*, 357–373. https://doi.org/10.1016/j.earscirev.2018.06.013

Del Grosso, S. J., Parton, W. J., Mosier, A. R., Hartman, M. D., Brenner, J., Ojima, D. S., & Schimel, D. S. (2001). Simulated interaction of carbon dynamics and nitrogen trace gas fluxes using the DAYCENT model. In *Modeling carbon and nitrogen dynamics for soil management* (pp. 303-332).

Del Grosso, S. J., Mosier, A. R., Parton, W. J., & Ojima, D. S. (2005). DAYCENT model analysis of past and contemporary soil N_2O and net greenhouse gas flux for major crops in the USA. *Soil and Tillage Research*, *83*(1), 9-24. https://doi.org/10.1016/j.still.2005.02.007

Del Grosso, S. J., Ogle, S. M., Parton, W. J., & Breidt, F. J. (2010). Estimating uncertainty in N_2O emissions from US cropland soils. *Global Biogeochemical Cycles*, *24*(1). https://doi.org/10.1029/2009GB003544

dos Reis Martins, M., Necpalova, M., Ammann, C., Buchmann, N., Calanca, P., Flechard, C. R., Hartman, M. D., Krauss, M., Le Roy, P., Mader, P., Maier, R., Thierry, M., Nicolardot, B., Skinner, C., Six, J., & Keel, S. G. (2022). Modeling N_2O emissions of complex cropland management in Western Europe using DayCent: Performance and scope for improvement. *European Journal of Agronomy*, *141*, 126613. https://doi.org/10.1016/j.eja.2022.126613

Eash, L., Berrada, A. F., Russell, K., & Fonte, S. J. (2021). Cover Crop Impacts on Water Dynamics and Yields in Dryland Wheat Systems on the Colorado Plateau. *Agronomy*, *11*(6), 1102. https://doi.org/10.3390/agronomy11061102

Fargione, J. E., Bassett, S., Boucher, T., Bridgham, S. D., Conant, R. T., Cook-Patton, S. C., Ellis, P. W., Falcucci, A., Fourqurean, J. W., Gopalakrishna, T., Gu, H., Henderson, B., Hurteau, M. D., Kroeger, K. D., Kroeger, T., Lark, T. J., Leavitt, S. M., Lomax, G., Mcdonald, R. I., … Griscom, B. W. (2018). Natural climate solutions for the United States. *Science Advances, 4*(11). https://doi.org/10.1126/sciadv.aat1869

García-González, I., Hontoria, C., Gabriel, J. L., Alonso-Ayuso, M., & Quemada, M. (2018). Cover crops to mitigate soil degradation and enhance soil functionality in irrigated land. *Geoderma*, *322*, 81-88. https ://doi.org/10.1016/j.geoderma.2018.02.024

Griscom, B. W., Adams, J., Ellis, P. W., Houghton, R. A., Lomax, G., Miteva, D. A., Schlesinger, W. H., Shoch, D., Siikamäki, J. V., Smith, P., Woodbury, P., Zganjar, C., Blackman, A., Campari, J., Conant, R. T., Delgado, C., Elias, P., Gopalakrishna, T., Hamsik, M. R., Herrero, M., Kiesecker, J., Landis, E., Laestadius, L., Leavitt, S. M., Minnemeyer, S., Polasky, S., Potapov, P., Putz, F. E., Sanderman, J., Silvius, M., Wollenberg, E. & Fargione, J. (2017) Natural climate solutions. *Proceedings of the National Academy of Sciences*, *114*(44), 11645-11650. https://doi.org/10.1073/pnas.1710465114

Guenet, B., Gabrielle, B., Chenu, C., Arrouays, D., Balesdent, J., Bernoux, M., Bruni, E., Caiman, J. P., Cardinael, R., Chen, S., Ciais, P., Desbois, D., Fouche, J., Frank, S., Henault, C., Lugato, E., Naipal, V., Nesme, T., Obersteiner, M., Pellerin, S., Powlson, D. S., Rasse, D. P., Rees, F., Soussana, J. F., Su, Y., Tian, H., Valin, H., & Zhou, F. (2021). Can N_2O emissions offset the benefits from soil organic carbon storage? *Global Change Biology*, *27*(2), 237-256. https://doi.org/10.1111/gcb.15342

Gurung, R. B., Ogle, S. M., Breidt, F. J., Williams, S. A., & Parton, W. J. (2020). Bayesian calibration of the DayCent ecosystem model to simulate soil organic carbon dynamics and reduce model uncertainty. *Geoderma*, *376*, 114529. https://doi.org/10.1016/j.geoderma.2020.114529

Han, Z., Walter, M. T., & Drinkwater, L. E. (2017). N_2O emissions from grain cropping systems: a meta-analysis of the impacts of fertilizer-based and ecologically-based nutrient management strategies. *Nutrient Cycling in Agroecosystems*, *107*(3), 335-355. https://doi.org/10.1007/s10705-017-9836-z

Hansen, J., Kharecha, P., Sato, M., Masson-Delmotte, V., Ackerman, F., Beerling, D. J., Hearty, P. J., Hoegh-Guldberg, O., Hsu, S. L., Parmesan, C., Rockstrom, J., Rohling, E. J., Sachs, J., Smith, P., Steffen, K., Van Susteren, L., von Schuckmann, K., & Zachos, J. C. (2013). Assessing "dangerous climate change": Required reduction of carbon emissions to protect young people, future generations and nature. *PLoS one*, *8*(12), e81648. https://doi.org/10.1371/journal.pone.0081648

IPCC. (2014). Climate Change 2014: Synthesis Report. The Fifth Assessment Report of Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change. https://www.ipcc.ch/report/ar5/syr/

IPCC. (2019). Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. Intergovernmental Panel on Climate Change. https://www.ipcc.ch/report/2019-refinement-to-the-2006-ipcc-guidelines-for-national-greenhouse-gas-inventories/

Kathage, J., Smit, B., Janssens, B., Haagsma, W., & Adrados, J. L. (2022). How much is policy driving the adoption of cover crops? Evidence from four EU regions. *Land Use Policy*, *116*, 106016. https://doi.org/10.1016/j.landusepol.2022.106016

Kaye, J. P., & Quemada, M. (2017). Using cover crops to mitigate and adapt to climate change. A review. *Agronomy for Sustainable Development*, *37*(1), 4. https://doi.org/10.1007/s13593-016-0410-x

Kladivko, E. J., Kaspar, T. C., Jaynes, D. B., Malone, R. W., Singer, J., Morin, X. K., & Searchinger, T. (2014). Cover crops in the upper midwestern United States: Potential adoption and reduction of nitrate leaching in the Mississippi River Basin. *Journal of Soil and Water Conservation*, *69*(4), 279-291. https://doi.org/10.2489/jswc.69.4.279

Lal, R. (2016). Beyond COP21: Potential and challenges of the “4 per Thousand” initiative. *Journal of Soil and Water Conservation*, *71*(1), 20A-25A. https://doi.org/10.2489/jswc.71.1.20A

Lassaletta, L., & Aguilera, E. (2015). Soil carbon sequestration is a climate stabilization wedge: Comments on Sommer and Bossio (2014). *Journal of Environmental Management*, *153*, 48-49. https://doi.org/10.1016/j.jenvman.2015.01.038

Lugato, E., Leip, A., & Jones, A. (2018). Mitigation potential of soil carbon management overestimated by neglecting N_2O emissions. *Nature Climate Change*, *8*(3), 219–223. https://doi.org/10.1038/s41558-018-0087-z

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

Mazzoncini, M., Sapkota, T. B., Barberi, P., Antichi, D., & Risaliti, R. (2011). Long-term effect of tillage, nitrogen fertilization and cover crops on soil organic carbon and total nitrogen content. *Soil and tillage research*, *114*(2), 165-174. https://doi.org/10.1016/j.still.2011.05.001

MCCC. (2020). *Cover crop decision tool.* Midwest Cover Crops Council. https://www.midwestcovercrops.org/covercroptool/

McClelland, S. C., Paustian, K., & Schipanski, M. E. (2021a). Management of cover crops in temperate climates influences soil organic carbon stocks: a meta-analysis. *Ecological Applications*, *31*(3), 1–19. https://doi.org/10.1002/eap.2278

McClelland, S. C., Paustian, K., Williams, S., & Schipanski, M. E. (2021b). Modeling cover crop biomass production and related emissions to improve farm-scale decision-support tools. *Agricultural Systems*, *191*. https://doi.org/10.1016/j.agsy.2021.103151

Muhammad, I., Sainju, U. M., Zhao, F., Khan, A., Ghimire, R., Fu, X., & Wang, J. (2019). Regulation of soil CO_2 and N_2O emissions by cover crops: A meta-analysis. *Soil and Tillage Research*, *192*, 103-112. https://doi.org/10.1016/j.still.2019.04.020

Murray, B. C., Sohngen, B., & Ross, M. T. (2007). Economic consequences of consideration of permanence, leakage and additionality for soil carbon sequestration projects. *Climatic change*, *80*(1), 127-143. https://doi.org/10.1007/s10584-006-9169-4

Ogle, S. M., Breidt, F. J., Easter, M., Williams, S., & Paustian, K. (2007). An empirically based approach for estimating uncertainty associated with modelling carbon sequestration in soils. *Ecological modelling*, *205*(3-4), 453-463. https://doi.org/10.1016/j.ecolmodel.2007.03.007

Ogle, S. M., Breidt, F. J., Easter, M., Williams, S., Killian, K. & Paustian, K. (2010). Scale and uncertainty in modeled soil organic carbon stock changes for US croplands using a process-based model. *Global Change Biology* 16(2): 810-822. https://doi.org/10.1016/j.ecolmodel.2007.03.007

Ogle, S. M., Butterbach-Bahl, K., Cardenas, L., Skiba, U., & Scheer, C. (2020). From research to policy: optimizing the design of a national monitoring system to mitigate soil nitrous oxide emissions. *Current Opinion in Environmental Sustainability*, *47*, 28-36. https://doi.org/10.1016/j.cosust.2020.06.003

Ogle, S.M., Kurz, W. A., Green, C., Brandon, A., Baldock, J., Domke, G., Herold, M., Bernoux, M., Chirinda, N., de Ligt, R., Federici, S., Garcia-Apaza, E., Grassi, G., Gschwantner, T., Hirata, Y., Houghton, R., House, J., Ishizuka, S., Jonckheere, I., Krisnawati, H., Lehtonen, A., Kinyanjui, M. J., McConkey, B., Naesset, E., Niinistö, S. M., Ometto, J. P., Panichelli, L., Paul, T., Petersson, H., Reddy, S., Regina, K., Rocha, M.T., Rock, J.,… McRoberts, R.

E. (2019). Chapter 2: General methodologies applicable to multiple land-use categories. In E. Calvo Buendia, K. Tanabe, A. Kranjc, J. Baasansuren, M. Fukuda, S. Ngarize, A. Osako, Y. Pyrozhenko, P. Shermanau, S. Federici (Eds). *2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories, Volume IV* (pp. 2.1-2.96). Intergovernmental Panel on Climate Change, Switzerland.

Ogle, S. M., Breidt, F. J., Del Grosso, S., Gurung, R., Marx, E., Spencer, S., Williams, S., Manning, D. (*in prep*). Counterfactual scenarios reveal historical impact of cropland management on soil organic carbon stocks in the United States.

Olson, K., Ebelhar, S. A., & Lang, J. M. (2014). Long-term effects of cover crops on crop yields, soil organic carbon stocks and sequestration. *Open Journal of Soil Science*, *4*(8). https://doi.org/10.4236/ojss.2014.48030

Parton, W. J., Hartman, M., Ojima, D., & Schimel, D. (1998). DAYCENT and its land surface submodel: description and testing. *Global and planetary Change*, *19*(1-4), 35-48. https://doi.org/10.1016/S0921-8181(98)00040-X

Piñeiro, G., Jobbágy, E. G., Baker, J., Murray, B. C., & Jackson, R. B. (2009). Set-asides can be better climate investment than corn ethanol. *Ecological Applications*, *19*(2), 277-282. https://doi.org/10.1890/08-0645.1

Piñeiro, V., Arias, J., Dürr, J., Elverdin, P., Ibáñez, A. M., Kinengyere, A., Opazo, C. M., Owoo, N., Page, J. R., Prager, S. D., & Torero, M. (2020). A scoping review on incentives for adoption of sustainable agricultural practices and their outcomes. *Nature Sustainability*, *3*(10), 809-820. https://doi.org/10.1038/s41893-020-00617-y

Plastina, A., Liu, F., Miguez, F., & Carlson, S. (2020). Cover crops use in Midwestern US agriculture: perceived benefits and net returns. *Renewable Agriculture and Food Systems*, *35*(1), 38-48. https://doi.org/10.1017/S1742170518000194

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

Post, W. M., & Kwon, K. C. (2000). Soil carbon sequestration and land-use change: processes and potential. *Global Change Biology*, *6*(3), 317-327. https://doi.org/10.1046/j.1365-2486.2000.00308.x

Powlson, D. S., Stirling, C. M., Jat, M. L., Gerard, B. G., Palm, C. A., Sanchez, P. A., & Cassman, K. G. (2014). Limited potential of no-till agriculture for climate change mitigation. *Nature Climate Change*, *4*(8), 678-683. https://doi.org/10.1038/nclimate2292

PRISM Climate Group. (2018). Oregon State University. https://prism.oregonstate.edu

Quemada, M., Lassaletta, L., Leip, A., Jones, A., & Lugato, E. (2020). Integrated management for sustainable cropping systems: Looking beyond the greenhouse balance at the field scale. *Global Change Biology*, *26*(4), 2584–2598. https://doi.org/10.1111/gcb.14989

R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Rockström, J., Gaffney, O., Rogelj, J., Meinshausen, M., Nakicenovic, N., & Schellnhuber, H. J. (2017). A roadmap for rapid decarbonization. *Science*, *355*(6331), 1269-1271. https://doi.org/10.1126/science.aah3443

Roe, S., Streck, C., Beach, R., Busch, J., Chapman, M., Daioglou, V., Deppermann, A., Doelman, J., Emmet-Booth, J., Engelmann, J., Fricko, O., Frischmann, C., Funk, J., Grassi, G., Griscom, B., Havlik, P., Hanssen, S., Humpenöder, F., Landholm, D., … Lawrence, D. (2021). Land-based measures to mitigate climate change: Potential and feasibility by country. *Global Change Biology, 27,* 6025-6058. https://doi.org/10.1111/gcb.15873

Schonbeck, M., & Morse, R. (2006). *Cover Crops for All Seasons.* Sustainable Agriculture Research and Education. *https://www.sare.org/resources/cover-crops-for-all-seasons/*

Simon, L. M., Obour, A. K., Holman, J. D., & Roozeboom, K. L. (2022). Long-term cover crop management effects on soil properties in dryland cropping systems. *Agriculture, Ecosystems & Environment*, *328*, 107852. https://doi.org/10.1016/j.agee.2022.107852

Soil Survey Staff. (2019). *Web soil survey.* U.S. Department of Agriculture, Natural Resources Conservation Service, Washington DC.

Sperow, M., Eve, M., & Paustian, K. (2003). Potential soil C sequestration on US agricultural soils. *Climatic Change*, *57*(3), 319-339. https://doi.org/10.1023/A:1022888832630

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2020). partR2: partitioning R2 in generalized linear mixed models. *bioRxiv*. https://doi.org/10.1101/2020.07.26.221168

United States of America. (2021). *The Long-Term Strategy of the United States; Pathways to Net-Zero Greenhouse Gas Emissions by 2050*. U.S. Department of State, Washington DC. https://www.whitehouse.gov/wp-content/uploads/2021/10/US-Long-Term-Strategy.pdf

US-EPA. (2022). *Inventory of U.S. greenhouse gas emissions and sinks: 1990–2018, Technical Bulletin No. 1957*. U.S. Environmental Protection Agency, Washington, DC.

USDA. (2022). *Biden-Harris Administration Announces Historic Investment in Partnerships for 70 Climate-Smart Commodities and Rural Projects*. U.S. Department of Agriculture. https://www.usda.gov/media/press-releases/2022/09/14/biden-harris-administration-announces-historic-investment

USDA-ERS. (1997). *Agricultural Resource Management Survey: Farm Financial and Crop Production Practices.* U.S. Department of Agriculture, Economic Research Service.

USDA-NASS. (2012). *2012 Census of Agriculture.* U.S. Department of Agriculture, National Agricultural Statistics Service. https://www.nass.usda.gov/AgCensus

USDA-NASS. (2017). *2017 Census of Agriculture.* U.S. Department of Agriculture, National Agricultural Statistics Service. https://www.nass.usda.gov/AgCensus

USDA-NASS. (2018). *Farm Production Expenditures Annual Summary*. U.S. Department of Agriculture, National Agricultural Statistics Service.

USDA-NASS. (June 2022). *Acreage.* U.S. Department of Agriculture, National Agricultural Statistics Service. https://downloads.usda.library.cornell.edu/usda-esmis/files/j098zb09z/0z70b374s/w9506686w/acrg0622.pdf

USDA-NRCS. (1997). National Resource Inventory. In N. R. C. Service (Ed.), Natural Resources Conservation Service. Statistical Laboratory, Iowa State University, Washington, DC.

USDA-NRCS. (2016). National Resources Inventory (NRI). U.S. Department of Agriculture, Natural Resources Conservation Service. https://www.nrcs.usda.gov/nri

USDA-NRCS. (2018). Effects of Conservation Practices on Water Erosion and Loss of Sediment at the Edge of the Field: A National Assessment Based on the 2003-06 CEAP Survey and APEX Modeling Databases. U.S. Department of Agriculture, Natural Resources Conservation Service.

Vicente-Vicente, J. L., García-Ruiz, R., Francaviglia, R., Aguilera, E., & Smith, P. (2016). Soil carbon sequestration rates under Mediterranean woody crops using recommended management practices: A meta-analysis. *Agriculture, Ecosystems & Environment*, *235*, 204-214. https://doi.org/10.1016/j.agee.2016.10.024

Wallander, S., Smith, D., Bowman, M., & Claassen, R. (2021). *Cover crop trends, programs, and practices in the United States* (No. 1476-2021-709). U.S. Department of Agriculture, Economic Research Service. *https://www.ers.usda.gov/webdocs/publications/100551/eib-222.pdf?v=5089.1*

Wang, J., Zhang, S., Sainju, U. M., Ghimire, R., & Zhao, F. (2021). A meta-analysis on cover crop impact on soil water storage, succeeding crop yield, and water-use efficiency. *Agricultural Water Management, 256,* 107085. https://doi.org/10.1016/j.agwat.2021.107085

White, K. E., Brennan, E. B., Cavigelli, M. A., & Smith, R. F. (2020). Winter cover crops increase readily decomposable soil carbon, but compost drives total soil carbon during eight years of intensive, organic vegetable production in California. *PLoS one*, *15*(2), e0228677. https://doi.org/10.1371/journal.pone.0228677

Williams, S.A., & Paustian, K. (2005). *Developing regional cropping histories for Century model U.S. National Level Simulations.* In A White Paper Submitted to the Environmental Protection Agency for Agricultural Soil Carbon Inventory Documentation.

CHAPTER 3: COVER CROP IMPACTS ON WATER DYNAMICS AND YIELDS IN DRYLAND WHEAT SYSTEMS ON THE COLORADO PLATEAU[1]

3.1 Introduction

Limited precipitation (180–300 mm yr^{-1}) has long challenged agriculture on the Colorado Plateau, which dates back to 800 AD. For example, in the year 1300, a multidecadal drought restricted maize cultivation and forced the abandonment of early settlements (Schwinning et al., 2008). Drought and low precipitation levels continue to limit agricultural production in the region. Dryland producers typically only grow one crop per two years and maintain their land under bare fallow in alternate years to minimize transpiration and accumulate soil water. While the traditional practice of fallowing land has been shown to recharge soil water, minimize crop failure, and stabilize yields (Smika, 1970), extended fallow periods leave the soil surface vulnerable to erosion and can result in the loss of soil organic matter (Blanco-Canqui et al., 2010). Estimates from a similar arid cropping system in New Mexico suggest that erosion on fallowed land can cause losses of more than 53 Mg ha^{-1} of topsoil per year, more than 97% of which was from wind erosion (Natural Resources Conservation Service, 2015), leading to soil fertility decline and regional air quality concerns. After centuries of agricultural activity, soils are shallow and degraded across much of the region, while climate models predict warmer, drier, and more variable conditions in the coming decades (Seager et al., 2007). Alternative management strategies are needed to address growing water limitations and soil degradation concerns and ensure the continued dryland crop production in the region, a vital component of the local economy (Alewoye Getie et al., 2020).

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Cover crops have been widely studied in more humid climates and have been shown to offer considerable promise for increasing soil water capture, reducing erosion, and improving soil fertility (Snapp et al., 2005; Schipanski et al., 2014). A recent review estimated that cover crops can increase soil organic carbon (SOC) stocks by 0.87 Mg ha^{-1} yr^{-1}, on average, and could potentially mitigate SOC losses after decades of fallow-based rotations and intensive tillage (Ruis & Blanco-Canqui, 2017). Cover crops have also been predicted to reduce erosion by 11–29% under future climate change scenarios (Miguez et al., 2016). Furthermore, by improving infiltration rates, cover crops may allow soils to better retain rainfall from intense storms and increase cropping system resilience in drought years (Basche & DeLonge, 2019). The potential benefits of cover crops are numerous and could help to address many of the challenges that dryland farmers on the Colorado Plateau are facing.

Despite the potential benefits offered by cover cropping, trade-offs are inevitable and cover crops can also compete with subsequent cash crops for water. For example, in the semiarid central Great Plains, a 46% reduction of soil water at wheat (*Triticum aestivum* L.) planting led to a wheat yield reduction of 36% when the typical 14-month fallow period was replaced with pea production (Nielsen & Vigil, 2017). In a review of cover crops in semiarid regions, Unger & Vigil (1998) noted that yield penalties for cash crops are common when cover crops replace a fallow period, particularly in the central-to-southern Great Plains where evapotranspiration rates are quite high relative to annual precipitation. The authors also emphasized the importance of early cover crop termination to give time for soil water to be replenished before planting of the subsequent cash crop.

The notion that cover crops in semiarid systems utilize already-scarce soil water and therefore impact yields has slowed their adoption in many semiarid regions. Similarly, research and experimentation with cover crops in these regions is lacking, particularly on the Colorado Plateau. As producers in the region look for solutions to reverse soil degradation, research is needed to evaluate the potential of cover crops to improve soil health and the magnitude of potential trade-offs for crop production. Improved understanding of how management factors such as tillage, cover crop species, and planting window can minimize crop yield penalties and maximize soil benefits would also improve adoption potential and increasing the viability of cover crops as a soil restoration practice.

To address this issue, we worked with local farmers and extension agents to develop two field trials to assess the potential of cover crops to mitigate soil degradation and examine trade-offs related to water use and crop yields. Data presented here represent preliminary findings from the first two cropping cycles of these experiments and focus on the short-term effects of cover crops in an environment representative of the Colorado Plateau. Our specific research objectives were to: (1) evaluate the early effects of cover crops vs. bare fallow on soil available N, water dynamics, and wheat yields; (2) assess the potential of different cover crop mixtures and planting windows on cover crop biomass production and associated effects on soil and crop yields; (3) understand how no-till vs. conventional tillage influences various cover crop performance metrics. We hypothesized that the incorporation of cover crops would result in a yield penalty in subsequent winter wheat, due to depleted soil water levels and altered available N dynamics. We further hypothesized that the implementation of no-till would offset the depletion of soil water due to cover crops, thus lessening the winter wheat yield penalty.

3.2 Methods

This research was conducted at the Southwestern Colorado Research Station near Yellow Jacket, CO (37°32′ N latitude; 108°44′ W longitude). At 2100 m in elevation, average monthly high temperatures vary between 3 and 31 °C (in January and July; respectively), and average precipitation is 400 mm yr^{-1}. Precipitation tends to be bimodal and occurs mostly during the winter months and during the monsoon season in late summer (U.S. Geological Survey, 2002). Soil at the research station is a Wetherill loam (fine-silty, mesic Aridic Haplustalfs; 36% sand, 41% silt, and 22% clay) (Soil Survey Staff, 2020; Berrada, 2012). Soils have a low organic matter content (1.4%) and a neutral pH of 6.9. Research trials were established on relatively flat, homogenous terrain.

The research considered here reports on two side-by-side field trials. Both were established on land that had been in conventionally-tilled, dryland rotations of dry bean (*Phaseolus vulgaris* L.), winter wheat, safflower (*Carthamus tinctorius* L.), and sunflower (*Helianthus annuus* L.) since 2010. Prior to 2010, fields were in irrigated alfalfa (*Medicago sativa* L.).

The first field trial (T1) was established in August 2015 to compare three winter wheat-cover crop rotations, which contained unique, fall-planted cover crop mixtures with a winter wheat-fallow control under no-till management. The winter wheat-fallow control is based on common local practices and involved a 10-month cycle of winter wheat (Sept-July) followed by a 14-month period of chemical fallow. The four treatments were established in 6 m × 61 m (372 m^2) plots in a randomized complete block (RCB) design with three replicate blocks.

In 2016, a second trial (T2) was added to provide additional insight into results observed in T1. This trial included additional treatment variables, such as cover crop planting window and tillage regime, to explore the effects of cover crop management on outcome measures. Eight different cover crop mixtures, including both spring- and fall-planted options, and a winter wheat-fallow control were established within two tillage regimes: no-till vs. conventional tillage. The experiment followed a split plot, RCB design with three replicate blocks, where whole plots (within each block) represented the two tillage treatments and subplots (3.7 m × 30.5 m) contained the cover crop and fallow treatments. We note that the two-year crop rotation alternated years between T1 and T2 to capture interannual variability and ensure that both crop phases were represented each year.

Discussions with local farmers, extension agents, and scientists from the National Resource Conservation Service served as a basis for cover crop mixture selection and cover crop seeding rates. Cover crop species were selected based on perceived drought tolerance and/or general interest of local farmers. Mixtures included varying proportions of grasses, legumes, and brassicas, and comprised between three and six species (Table 3.1). Seeding rates were determined based on cost, growth traits, desired expression of each species, and experience of the stakeholders involved.

In both trials, all treatments followed a two-year rotation in accordance with local practices. Fall-planted cover crops were established in late August or September and terminated in June. Spring-planted cover crop treatments were left to fallow until planting of cover crops in April, such that cover crops were only allowed to grow for roughly 2 months before termination in June. Control treatments were maintained as weed-free fallow (using herbicides or tillage, depending on the treatment) from study establishment in August until wheat planting in September of the following year, thus representing the traditional wheat-fallow rotation in the region (14 months fallow and 10 months in winter wheat). In all treatments, the hard red winter wheat variety 'Fairview' was planted in September (3 months after cover crop termination) and harvested the following June or July (Table 3.2). Winter wheat was planted at a rate of 56 kg ha^{-1} with rows spaced approximately 21 cm.

Chemical weed control and cover crop termination depended on tillage regime. For all plots in T1 and no-till plots in T2, weeds were controlled and cover crops were terminated using a mixture of glyphosate (*N*-(phosphonomethyl)glycine) and 2,4-D amine (2,4-

dichlorophenoxyacetic acid). Weeds were controlled prior to cover crop and winter wheat seeding and in the spring in fallow plots. For T2 plots under conventional tillage, cover crop termination and weed control were done mechanically; plots were disked with three passes at the start of the trial (Fall 2016), spring-planted cover crop treatments were chisel-plowed and cultivated prior to planting (April 2017), cover crops were terminated using a tandem disk (June 2017), and a field cultivator with sweep attachments was used prior to winter wheat planting. In accordance with local practices, no fertilizer was added to either trial throughout the duration of the study.

Cover crop biomass in both trials was evaluated in each plot just before cover crop termination (dates presented in Table 3.2) using a 75 cm diameter range hoop placed near the center of each plot. Biomass within the hoop was cut at a height of 2-4 cm above the soil surface, oven-dried at 60 °C for 72 h and weighed.

Wheat yield data were collected using a Hege plot combine (1.2 m width) shortly after wheat plants reached grain maturity (BBCH 89; Table 3.2). Wheat was harvested from subplots in the center of each treatment plot (six rows in width and approximately 58 m and 28 m in length in T1 and T2, respectively) to avoid edge effects. Wheat from each plot was cleaned using an electric winnower, weighed, and tested for moisture and density. Wheat yields were adjusted to a water content of 11%. A subsample of wheat grain from each plot was analyzed for grain protein content using a LECO N combustion analyzer.

Soil samples (0–15 cm) were taken prior to winter wheat planting each year using a soil probe and air-dried and 2 mm sieved upon return to the lab. In 2016 and 2017, soil nitrate was measured in a subset of treatments for each trial due to limited resources, namely cover crop mixtures 1 and 2 and the fallow in T1 and mixtures 1, 5, 6, and 8, and the fallow in conventionally tilled and no-till plots in T2. Soil samples were sent to Ward Laboratories in Kearney, NE for analysis, where nitrate was measured using a flow injection analyzer. In 2018 (after receiving additional funding), all treatments in T1 were sampled and extracted for NO_3-N at Colorado State University with 2*M* KCl following methods detailed in Keeney & Nelson (1983). NO_3-N concentrations were determined using vanadium (III) chloride as a reduction agent and with an automated colorimeter (Shimadzu Scientific Instruments, Japan).

Gravimetric soil water was measured each year 1–2 weeks prior to winter wheat planting. All treatments in T1 were sampled in 2016 and 2018, while in T2 a subset of treatments (mixtures 1, 5, 6, 8, and fallow in both conventional and no-till) were evaluated in 2017. Soil was sampled in 30 cm increments using a tractor-mounted Giddings hydraulic probe. While we targeted a sampling depth of 180 cm, in most cases the probe was not able to reach this depth due to drought conditions, indicating a lack of available soil moisture below the sampling depth. For this reason, soil was sampled to a depth of 90 cm, which was considered to be representative of the water available to the growing wheat roots. In 2016 and 2017, one subsample core was collected per plot, while in 2018 two cores per plot were collected and composited for soil water determination. Soils were weighed, dried at 105 °C in a forced air convection oven for 48 h, and reweighed to determine their gravimetric water content. Gravimetric water content was converted to volumetric water by multiplying by the bulk density for the sampling depth (1.35 g cm^{-3} for 0–30 cm depth, 1.40 g cm^{-3} for 30–60 cm depth, and 1.45 g cm^{-3} for the 60–90 cm depth; based on unpublished data from the study site). To determine soil water storage throughout the soil profile (0–90 cm), we calculated the sum of soil water storage for each layer (volumetric soil water content multiplied by depth).

Cover crop production, wheat yields, soil nitrate, and soil water storage were analyzed using a multifactor analysis of variance (ANOVA). Assumptions of ANOVA (normality and homogeneity of variance) were verified, and no transformations were required. For T1, cover crop treatment was included as a fixed effect and block was included as a random effect. For T2, the main and interactive effects of tillage regime and cover crop treatment were included as fixed effects, and block and block × tillage subplots were included as random effects. Differences among treatments were estimated using Tukey-adjusted pairwise comparisons, generated by the emmeans package in R (Lenth et al., 2018).

Since few differences were observed between cover crop mixtures, additional comparisons were focused on presence or absence of cover crops (T1) and cover crop planting window (T2) using orthogonal contrasts. In T1, mixtures 1, 2, and 3 were grouped together and compared against the fallow control. In T2, fall-planted mixtures (1–5) and spring-planted mixtures (6–8) were grouped separately and compared with one another and the fallow treatment, averaged across tillage regimes.

Wheat yield penalty was also calculated based on these groupings for descriptive purposes; wheat yield penalty was estimated in T1 by comparing cover crop treatments against the fallow control in 2016 and 2018. In T2, wheat yield penalty was calculated and averaged for all fall-planted cover crop treatments and separately averaged for all spring-planted cover crop treatments. Yield penalty was calculated by using the following equation:

$$Yield\ penalty = 1 - \frac{Yield_{Fallow} - Yield_{Cover\ Crop}}{Yield_{Fallow}}$$

We examined relationships between cover crop biomass (in all treatments) and soil nitrate, soil water storage in the top 90 cm of the soil profile, and subsequent wheat yields using linear regression. The slopes of these linear regressions were used to estimate amount change in soil nitrate and water storage per unit change in cover crop biomass. All analyses were conducted using R statistical software (R Core Team, 2013).

3.3 Results

3.3.1 Cover crop biomass

Average cover crop biomass in Trial 1 varied by year due to interannual variability in precipitation quantity and distribution (Table 3.3). Average cover crop biomass for all mixtures was 5020 ± 418 kg ha^{-1} in 2016 and only 1510 ± 110 kg ha^{-1} in 2018, after a severe drought that the region experienced in late 2017 and early 2018. Above-ground biomass production was similar for all cover crop mixes in both 2016 and 2018 ($p > 0.05$; Table 3.4).

In T2, the planting window of the cover crop affected total biomass, with fall-planted mixtures producing more total biomass than spring-planted mixtures ($p < 0.001$; Table 3.5). Within planting window, however, mixtures did not differ in terms of total biomass produced ($p > 0.05$). Tillage regime also did not have a significant effect on total cover crop biomass ($p > 0.05$; Table 3.5).

3.3.2 Winter wheat yields

Winter wheat yields also varied by year, with grain production for the control (fallow) averaging 4.03 Mg ha^{-1} in 2017 and 1.35 Mg ha^{-1} in 2019 (Table 3.4). Cover crop treatments affected wheat grain yields in both 2017 and 2019 ($p < 0.001$ and $p = 0.028$, respectively). When treatments were grouped according to the presence or absence of cover crops for calculation of yield penalty and for orthogonal contrast grouping, wheat yields in cover crop treatments averaged 2.94 Mg ha^{-1} in 2017, making the wheat yield penalty 27% on average. In 2019, no wheat yield penalty was observed as compared to the fallow control due to the relatively low amount of cover crop biomass produced in 2018. In fact, cover crop treatments yielded on average 1.52 Mg ha^{-1}, or 13% higher than the fallow in 2019 ($p = 0.008$; Table 3.4). Wheat protein (grain N concentration) was greater in the fallow treatment as compared to cover crop treatments in 2017 when analyzed using orthogonal contrasts ($p = 0.023$) but did not differ in 2019.

Overall, 2018 wheat yields in T2 were very low, averaging only 1.27 Mg ha^{-1} in the fallow treatment, following the severe drought experienced during the wheat growing season (Table 3.3, 3.5). Yields depended on cover crop planting window ($p < 0.001$), with fallow control yielding the highest, fall-planted cover crop treatments yielding the lowest, and spring-planted cover crops resulting in intermediate yields (Table 3.5). The wheat yield penalty was on average 78% lower than the fallow in the fall-planted cover crop plots and 22% lower for the spring-planted plots.

Regression analyses indicated that wheat yield was inversely correlated with cover crop biomass produced the year prior, as evidenced by the linear regression between 2017 cover crop biomass and 2018 wheat yields (Fig. 3.1; $R^2 = 0.53$; $p < 0.001$). For every 1000 kg ha^{-1} of cover crop biomass produced, subsequent wheat yields declined by 0.20 Mg ha^{-1}. Though wheat yields in conventionally tilled treatments tended to be lower than in no-till treatments, tillage regime had no significant effect on wheat yields ($p > 0.05$; Table 3.5).

3.3.3 Soil water and soil nitrate

In 2016, following substantial cover crop biomass production, soil water storage, and soil nitrate levels at wheat planting were both consistently lower in cover crop treatments as compared to fallow plots (Table 3.4; Fig. 3.2). However, no differences in soil water content were detected throughout the soil profile at 2018 wheat planting ($p > 0.05$; Fig. 3.2) following low cover crop biomass production.

In T2, soil water and soil nitrate levels at 2017 wheat planting were both lowest in fall-planted cover crop treatments, highest in the fallow, and intermediate for the spring-planted treatments (Table 3.5; Fig. 3.3). Similar to the relationship between wheat yield and cover crop biomass (Fig. 3.1), soil water storage and soil nitrate levels were also negatively correlated with cover crop biomass production ($R^2 = 0.55$ and $R^2 = 0.38$, respectively; Fig. 3.4). Linear regression equations show that soil water storage in the top 90 cm of soil decreased by 10 mm and soil nitrate decreased by 0.39 mg kg^{-1} for every 1000 kg ha^{-1} of cover crop biomass produced. Tillage regime had no significant effect on soil nitrate and soil water levels (Table 3.5). Through regression analysis, reductions in soil water and soil nitrate levels following cover crop growth were further correlated with subsequent wheat yields ($R^2 = 0.459$ and $R^2 = 0.457$, respectively; Fig. 3.4). Wheat yields were reduced by 0.126 Mg ha^{-1} per cm of soil water depletion following cover crops and by 0.165 Mg ha^{-1} per mg kg^{-1} of soil nitrate immobilized by cover crops.

3.4 Discussion

3.4.1 Cover crop biomass production and trade-offs with wheat yields

Cover crop biomass during the study period varied considerably year to year, largely according to precipitation (Table 3.3), such that the treatments averaged 1511 kg ha^{-1} in 2017, following drought, but reached 5024 kg ha^{-1} in 2016, a year with greater precipitation. We note that these values were largely in the range observed for other dryland wheat-based systems. For example, Kelly et al. (2021) reported an average cover crop biomass of 3304 kg ha^{-1} from ten study sites in the Central Great Plains. Nielsen et al. (2015), also working in Eastern Colorado, reported cover crop biomass production to range from 1366 to 5880 kg ha^{-1}, depending on available growing season water, cover crop species, and plant stands. However, unlike the research presented by Nielsen et al. (2015), biomass did not vary among cover crop mixtures within the same year and planting window. While species richness has often been linked with aboveground productivity (Tilman et al., 2001), studies exploring effects of cover crop diversity have shown that productivity can be more dependent on the presence of a highly productive species, and diverse species mixtures will not necessarily produce more biomass than a highly productive monoculture (Finney et al., 2016; Florence et al., 2019). Growing cover crops in mixtures has been shown to have little to no effect on water use efficiency (Nielsen et al., 2015), which is most likely the greatest limitation in the study region. Furthermore, in the present research, the species seed in the mixtures were not always expressed in the established cover crop stands due to drought and competition from volunteer wheat, which may have limited species effects to some extent.

Winter wheat yields also varied by year, with yearly averages for the control (fallow) treatments ranging from 1.4 to 4.0 Mg ha^{-1} (Table 3.4). The 1.4 Mg ha^{-1} average was from 2018 in T2, after a severe drought year on the Colorado Plateau (Table 3.3). Averages from Southwest Colorado for winter wheat according to 2016–2019 USDA census data were 25.6 bu acre^{-1}, or 1.7 Mg ha^{-1}, suggesting that yields were within expected range for the study period (Colorado Agricultural Statistics Service, 2018, 2019, 2020).

Dryland wheat yields in semiarid climates have been shown to be lower when grown after a cover crop as compared to after a fallow period. The wheat yield penalty was on average 27% in 2017. This is on par with the average yield penalty observed in similar semiarid environments; Nielsen & Vigil (2005) observed an average 26.2% yield penalty in dryland wheat plots following a legume cover crop in a 6-year study conducted in eastern Colorado, and a separate study from Nielsen et al. (2016) demonstrated a yield penalty, which ranged from 3 to 40% following cover crop treatments. Nielsen et al. (2016) compiled a summary table showing that change in wheat yield following cover crops in dryland systems can range from a reduction of 79% to an increase of 5%, similar to what was observed in our study. More severe yield penalties tended to occur in semiarid climates, dryland cropping systems, and when wheat yields following fallow periods were also exceptionally low due to drought. This may explain why, after the drought in 2018, the yield penalty following fall-planted cover crops was 78%, much higher than for other years.

While wheat yield penalties were affected by planting window, cover crop species mixture within the same planting window and growing season did not affect subsequent wheat yields. This is unsurprising as cover crop species (legumes in particular) were not strongly expressed in cover crop stands. Furthermore, cover crop diversity has been shown to increase ecosystem services such as weed suppression, N retention, and aboveground biomass N, but is not typically associated with effects on cash crop yield, at least in the short-term (Finney & Kaye, 2017; Kumar et al., 2020). Differences in wheat yields following cover crops of different planting windows (planted in the spring or in the previous fall) were observed, with a wheat yield reduction averaging 78% following fall-planted cover crops and 22% following spring-planted cover crops. This effect was directly related to the difference in biomass in fall- and spring-planted treatments; fall-planted treatments averaged 3850 kg ha^{-1} whereas spring-planted treatments only produced on average 781 kg ha^{-1}. The relatively strong inverse correlation between cover crop biomass produced and subsequent wheat yields (R^2= 0.53; Fig. 3.1) emphasizes the importance of regulating cover crop biomass to minimize the cash crop yield penalty. A similar finding was reported by Holman et al. (2018), in which cover crop species that produced the least amount of biomass resulted in lower wheat yield penalties. Unger & Vigil (1998) concluded that the timely termination of cover crops is essential, particularly in semiarid environments, to prevent excessive water uptake by cover crops and ensure sufficient soil water recharge. Nielsen & Vigil (2005) showed that earlier termination of legume cover crops was negatively correlated with subsequent wheat yield; cover crops terminated in early June reduced wheat yields by only 23%, whereas cover crops terminated in late July reduced wheat yields by 42%.

Spring-planted cover crops therefore may be a way to gain benefits associated with cover crops while minimizing yield penalty. Alternatively, to limit cash crop yield penalty, fall-planted cover crops could be terminated early to limit cover crop biomass (Miller et al., 2006), while still providing soil cover through the fall, thus protecting against erosion and suppressing weeds (Kumar et al., 2020; Baraibar et al., 2018). It is important to note, however, that restricting cover crop biomass is likely to decrease potential soil health benefits, such as building SOC, soil fertility restoration and erosion control (Finney et al., 2016). Additional research is needed to better elucidate the trade-offs between cover crop biomass production, soil benefits, and cash crop yield penalties.

3.4.2 Available soil N and soil water as drivers of wheat yield

The yield penalty associated with cover crops is typically attributed to lower soil water and/or available N at cash crop planting following cover crops, evidenced by the strong correlation of these factors with wheat yields (Fig. 3.4). Decreased water availability is widely understood to contribute to yield penalties following cover crops in semiarid regions due to increased evapotranspiration during cover crop growth (Lyon et al., 2007; Nielsen et al., 1999; Nielsen et al., 2002). Nielsen & Vigil (2005) compared fertilized fallow plots with legume cover crops and found similar available N levels at wheat planting, but a decrease in wheat yield of 15.2 kg ha^{-1} for every mm less available soil water in the top 1.8 m due to legume production. Schlegel & Havlin (1997) similarly reported that every millimeter of soil water depleted following a hairy vetch cover crop resulted in a reduction of wheat yields by 15 kg ha^{-1}. A comparable decrease in wheat yield of 12.6 kg ha^{-1} per mm loss of available soil water was observed in the present study. Though different soil depths were utilized between our calculations, sampling any further was prohibited by impenetrability of the soil at depth, indicating that soil water was extremely scarce below 0.9 m and likely would not contribute much to crop water use. The correlation between soil water and wheat yield (Fig. 3.4) provides evidence that the 0.20 Mg ha^{-1} decrease in wheat yields associated with every 1000 kg ha^{-1} increase in cover crop biomass was attributed in part to the depletion of available soil water.

No-till management has been shown to conserve soil water through enhanced infiltration, increased snow catch, and reduced evaporation, and can help ameliorate water depletion following cover crops (Burgess et al., 2014). However, these effects are typically observed after several cover crop cycles, and in this relatively short timeframe tillage regime had no effect on wheat yields, cover crop biomass, and soil parameters.

Though reductions in available water undoubtedly contribute to the yield penalty observed in cover crop plots, lower available N was also observed at wheat planting following cover crops (Table 3.4, 3.5; Fig. 3.4) and could have been a colimiting factor for grain production. This effect is likely due to "preemptive competition", a concept coined by Thorup-Kristensen (1993), meaning that N assimilated by cover crops was not mineralized back into the soil in time to be utilized by subsequent cash crops. Preemptive competition is more likely in arid regions, where biotic decomposition is limited by a lack of moisture (Helgason et al., 2014). After the first cycle of cover crops in T1, soil nitrate at wheat planting was lower following cover crops (9.93 mg kg^{-1}) than following the 14-month fallow period (17.1 mg kg^{-1}), and wheat grain protein content was also lower following cover crops than after the fallow treatment (Table 3.4). In 2018, following the drought year, which produced very little cover crop biomass, N levels at wheat planting were still lower in cover cropped plots (12.4 mg kg^{-1}) than in fallow plots (19.4 mg kg^{-1}). This suggests that N assimilated by cover crops in previous cycles had not yet been mineralized back into plant-available N. Still, there was no winter wheat yield penalty in 2018, suggesting that perhaps soil water limitations were greater in this year, alleviating the limitation of plant-available N.

In T2, soil nitrate at wheat planting was inversely correlated with cover crop biomass produced in the previous cycle (Fig. 3.4); soil nitrate decreased by 0.39 mg kg^{-1} for every 1000 kg ha^{-1} of cover crop biomass produced. Fall-planted mixtures not only produced more biomass, but also appeared to have a lower proportion of legumes (personal observation), and would thus be likely to have a higher C:N ratio and immobilize more N. Thomas et al. (2017) compared soil nitrate and spring wheat yields following differing amounts of cover crop biomass and found similar relationships; greater cover crop biomass production was correlated with lower soil nitrate levels and greater wheat yield penalties. For an environment such as the Colorado Plateau, with low yearly precipitation and largely unfertilized dryland cropping systems, the immobilization of N and slow decomposition rates could contribute to a large trade-off of cash crop productivity. Early termination might be key in these systems to limit biomass production and allow more time before cash crop planting for residues to decay. A small N fertilizer input, or better expression of legumes in cover crop mixtures could potentially overcome diminished N availability following cover crop growth.

Despite large yield penalties in wheat grown in 2016–2018, no yield penalty was observed in winter wheat harvested in 2019, following the 2018 drought and very low cover crop biomass production. Cover crop treatment plots actually yielded on average 13% higher than the fallow control in 2019 ($p = 0.008$; Table 3.4). In dryland systems, effects of a cover crop grown in year 1 of a rotation could have impacts on yield of subsequent crops in years 2, 3, and 4 of a rotation, particularly when precipitation is below average for the region (Kirkegaard & Ryan, 2014). However, in the present research, sparse biomass production in cover crop plots allowed soil water to catch up to the levels observed in fallow plots by 2018 wheat planting, and no difference between soil water in cover crop and fallow treatments was observed ($p > 0.05$; Table 3.4). Equivalent soil water levels following fallow and cover crop growth could also be due to an effect described by Nielsen et al. (2016), where in some of the site-years slight water consumption by cover crops was offset by increased precipitation storage due to soil cover. Generally, retention of plant biomass and soil cover have been shown to reduce soil evaporation and contribute to increased soil water recharge (Kelly et al., 2021; Ranaivoson et al., 2017). The increase in wheat yields following cover crops in this year could also be due to slight soil health benefits, such as increased biological activity, aggregation, or organic matter content (Schipanski et al., 2014), which could contribute to increased wheat yields over time.

3.5 Conclusions

In semiarid, dryland systems such as those on the Colorado Plateau, cover crops can negatively impact cash crop productivity, presenting a trade-off in terms of productivity and soil health. Data from the field experiments presented here clearly highlight this trade-off, as wheat yield penalties following cover crop growth were as great as 78%, depending on the year and planting window of cover crops. Wheat yield penalty, soil nitrate levels at wheat planting, and soil water at wheat planting all were inversely correlated with cover crop biomass produced in the preceding season, indicating that the yield penalty is attributed to reductions in soil water and available N. As fall-planted mixtures produced significantly more biomass than spring-planted mixtures, spring cover crop planting or earlier termination of fall-planted cover crops could prevent excessive soil water use and minimize yield penalties to the subsequent crop. The trade-off in cash crop productivity presented here may not be observed in more humid climates or in irrigated systems, where moisture is not limiting. Further research is needed to evaluate the impact of cover crops on dryland wheat yields over time and whether the potential long-term benefits to soil health and water use efficiency are worth the trade-off in cash crop productivity.

CHAPTER 3 TABLES AND FIGURES

Table 3.1. Cover crop mixtures planted in Trial 1 and 2 at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Species are listed followed by percent contribution (by seed weight) in parenthesis.

Trial 1

Cover Crop Treatment	Cover Crop Seeding Rate (kg ha^{-1})	Species
Mix 1	32.4	Hairy Vetch [a] (14%), Yellow Sweet Clover [b] (3%), Winter Pea [c] (83%)
Mix 2	39.5	Hairy Vetch (8%), Yellow Sweet Clover (2%), Winter Pea (48%), Winter Rye [d] (43%)
Mix 3	31.7	Hairy Vetch (6%), Yellow Sweet Clover (1%), Winter Pea (34%), Winter Rye (53%), Winfred Turnip [e] (3%), Winter Canola [f] (3%)
Fallow	NA [u]	Fallow-Control

Trial 2

Cover Crop Treatment	Planting Window	Cover Crop Seeding Rate (kg ha^{-1})	Species
Mix 1	Fall	27.7	Winter Pea (54%), [g] Berseem Clover (4%), Yellow Sweet Clover (1%), [h] Winter Barley (35%), [i] Forage Radish (2.5%), [j] Forage Turnip (2.5%)
Mix 2	Fall	37.1	Winter Pea (55%), [k] Lentil (5%), Winter Barley (37%), Winter Canola (3%)
Mix 3	Fall	48.2	Winter Pea (50%), Hairy Vetch (10%), Winter Barley (17%), Forage Radish (3%), Winter Canola (3%)
Mix 4	Fall	39.3	Winter Pea (59%), Hairy Vetch (19%), Winter barley (7.5%), [l] Winter Oat (7.5%), Forage Radish (2.5%), Winter Canola (2.5%)
Mix 5	Fall	42.6	Winter Pea (31%), Hairy Vetch (8%), Winter Barley (27%), Oats (27%), Forage Radish (3%), Winter Canola (3%)
Mix 6	Spring	27.2	Hairy Vetch (14%), [m] Spring Pea (62%), [n] Spring Oat (16%), Winter Canola (1%), [o] Flax (2%), [p] Safflower (4%)
Mix 7	Spring	11.2	[q] Balansa Clover (40%), [r] Crimson Clover (20%), [s] Ryegrass (30%), Forage radish (10%)
Mix 8	Spring	25.1	Crimson Clover (2%), Spring Pea (67%), [t] Spring Barley (25%), Forage radish (7%)
Fallow	NA	NA	Fallow-Control

[a] *Vicia villosa* R. [b] *Melilotus officinalis* L. [c] *Pisum sativum* L. [d] *Secale cereale* L. [e] *Brassica napus* L., *cv.* Winfred [f] *Brassica napus* L. [g] *Trifolium alexandrinum* L. [h] *Hordeum vulgare* L. [i] *Raphanus sativus* L. [j] *Brassica rapa* L. [k] *Lens culinaris* L. [l] *Avena sativa* L. [m] *Pisum sativum* L. [n] *Avena sativa* L. [o] *Linum usitatissimum* L. [p] *Carthamus tinctorius* L. [q] *Trifolium michelianum* L. [r] *Trifolium incarnatum* L. [s] *Lolium perenne* L. [t] *Hordeum vulgare* L. [u] NA, not applicable.

Table 3.2. Planting, cover crop termination and winter wheat harvest dates for Trials 1 (T1) and 2 (T2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado.

	Trial	
	T1	T2
Cover Crop Cycle 1 Planting Date	Sept. 28, 2015	Fall-planted: Aug. 11, 2016 Spring-planted: Apr. 13, 2017
Cover Crop Cycle 1 Termination Date	June 10, 2016	June 20, 2017
Winter Wheat Cycle 1 Planting Date	Sept. 19, 2016	Sept. 21, 2017
Winter Wheat Cycle 1 Harvest Date	June 20, 2017	July 25, 2018
Cover Crop Cycle 2 Planting Date	Aug. 30, 2017	NA [1]
Cover Crop Cycle 2 Termination Date	June 8, 2018	NA
Winter Wheat Cycle 2 Planting Date	Sept. 28, 2018	NA
Winter Wheat Cycle 2 Harvest Date	Aug. 7, 2019	NA

[1] NA, not applicable. Complete data for cover crop-wheat cycle not available for this trial at the time of manuscript preparation.

Table 3.3. Monthly average temperature (T) and precipitation (P) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado during the experimental period. Total P throughout growing season (defined as August–July) is displayed in italics.

	Growing Season							
	2015–2016		2016–2017		2017–2018		2018–2019	
Month	T	P	T	P	T	P	T	P
	°C	mm	°C	mm	°C	mm	°C	mm
August	20.8	26.9	18.8	45.0	20.0	14.5	21.2	6.6
September	18.1	25.4	15.7	26.4	16.1	44.7	18.1	27.9
October	11.8	36.6	12.1	1.5	10.4	1.0	8.7	60.5
November	1.9	34.5	5.7	27.7	7.5	2.5	2.3	5.8
December	−3.1	11.2	−1.6	40.9	2.2	0.3	−2.4	15.2
January	−4.3	23.4	−2.7	36.8	0.9	15.2	−3.8	22.9
February	NA [1]	NA	2.2	21.3	0.7	9.9	−4.2	23.4
March	5.0	6.4	6.6	17.8	4.1	4.8	2.9	55.9
April	7.3	15.7	7.2	11.2	9.4	8.4	9.2	27.4
May	11.2	33.8	11.8	27.4	14.7	21.6	9.1	47.8
June	20.8	1.5	20.3	0.0	20.2	8.6	17.2	22.9
July	21.7	61.5	21.7	58.4	22.5	27.2	21.2	6.6
Total P		*276.9*		*314.4*		*158.7*		*316.3*

[1] NA, weather station data not available.

Table 3.4. Mean cover crop biomass, soil nitrate, wheat yield, and wheat protein values from two cropping cycles of a field trial (T1) located at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Values with different lowercase letters (by column) indicate differences to an alpha level of 0.05, as determined by Tukey-adjusted multiple comparisons.

	2016–2017 Cycle					2018–2019 Cycle				
Cover Crop Treatment	Cover Crop Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield	Wheat Protein	Cover Crop Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield	Wheat Protein
	kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}	% Crude Protein	kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}	% Crude Protein
Mix 1	4560 ^{a}	9.68 ^{a}	157 ^{a}	3.00 ^{a}	13.7 ^{a}	1410 ^{a}	13.6 ^{ab}	129 ^{a}	1.57 ^{a}	10.0 ^{a}
Mix 2	4860 ^{a}	10.2 ^{a}	151 ^{a}	3.01 ^{a}	13.7 ^{a}	1570 ^{a}	11.0 ^{a}	120 ^{a}	1.47 ^{ab}	9.2 ^{a}
Mix 3	5650 ^{a}	NE [1]	163 ^{a}	2.82 ^{a}	14.0 ^{a}	1550 ^{a}	12.7 ^{a}	119 ^{a}	1.51 ^{ab}	8.7 ^{a}
Fallow	NE	17.1 ^{b}	224 ^{b}	4.03 ^{b}	15.0 ^{a}	NE	19.4 ^{b}	131 ^{a}	1.35 ^{b}	8.8 ^{a}
ANOVA [2]										
	0.60	0.005	0.010	<0.0001	0.099	0.60	0.013	0.38	0.028	0.097
Orthogonal Contrasts [3]										
	NA [4]	0.002	0.003	<0.0001	0.023	NA	0.0028	0.23	0.008	0.26

[1] NE, not evaluated. [2] Differences analyzed using a multifactor analysis of variance (ANOVA) with treatment included as a fixed effect and block included as a random effect. *p*-values presented. [3] Orthogonal contrast analyses performed to evaluate the effect of presence of cover crop vs. fallow control. *p*-values presented. [4] NA, not applicable.

Table 3.5. Cover crop biomass, soil nitrate, soil water, and wheat yield data from the 2016-2018 cropping cycle of a field trial (T2) located at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Treatments are either a cover crop-winter wheat or fallow-winter wheat rotation. Values with different lowercase letters (by column) indicate differences to an alpha level of 0.05, as determined by Tukey-adjusted multiple comparisons.

Cover Crop Planting Window	Cover Crop Treatment	Tillage	Cover Crop Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield
			kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}
Fall	Mix 1	CT[1]	3620^{ab}	3.28^{a}	149^{abc}	0.11^{ab}
		NT[2]	4120^{a}	4.57^{ab}	147^{ab}	0.42^{abcd}
	Mix 2	CT	3570^{ab}	NE[3]	NE	0.08^{a}
		NT	3940^{a}	NE	NE	0.34^{abcd}
	Mix 3	CT	3660^{ab}	NE	NE	0.22^{abc}
		NT	3840^{a}	NE	NE	0.41^{abcd}
	Mix 4	CT	4490^{a}	NE	NE	0.14^{abc}
		NT	3520^{ab}	NE	NE	0.59^{abcde}
	Mix 5	CT	3760^{ab}	3.01^{a}	137^{a}	0.15^{abc}
		NT	3970^{a}	4.16^{ab}	151^{ab}	0.42^{abcd}
Spring	Mix 6	CT	855^{c}	5.39^{b}	177^{abcd}	0.77^{abcdef}
		NT	742^{c}	5.37^{b}	173^{abcd}	1.00^{bcdef}
	Mix 7	CT	556^{c}	NE	NE	0.81^{bcdef}
		NT	440^{c}	NE	NE	1.24^{ef}
	Mix 8	CT	1120^{bc}	5.30^{b}	194^{bcd}	0.84^{cdef}
		NT	728^{c}	5.48^{b}	185^{abcd}	1.33^{f}
	Fallow	CT	NE	9.15^{c}	211^{d}	0.95^{def}
		NT	NE	9.61^{c}	209^{cd}	1.60^{f}
				ANOVA [4]		
Source of variation						
Cover Crop Treatment			<0.001	<0.001	<0.001	<0.001
Tillage Treatment			1.00	0.129	0.880	0.102
Cover Crop x Tillage			0.86	0.377	0.740	0.738
				Orthogonal Contrasts [5]		
Planting Window Comparison						
Fall- vs. spring-planted			<0.001	<0.001	0.201	<0.001
Fall-planted vs. fallow			NA	<0.001	<0.001	<0.001
Spring-planted vs. fallow			NA	<0.001	<0.001	0.014

[1] CT, conventionally tilled. [2] NT, no-till. [3] NE, not evaluated. [4] Differences analyzed using a multifactor analysis of variance (ANOVA) with cover crop treatment and tillage included as fixed effects and block and tillage split-plots included as random effects. *p*-values presented. [5] Orthogonal contrast analyses performed to detect differences between fall- and spring-planted cover crop treatments and fallow control. *p*-values presented.

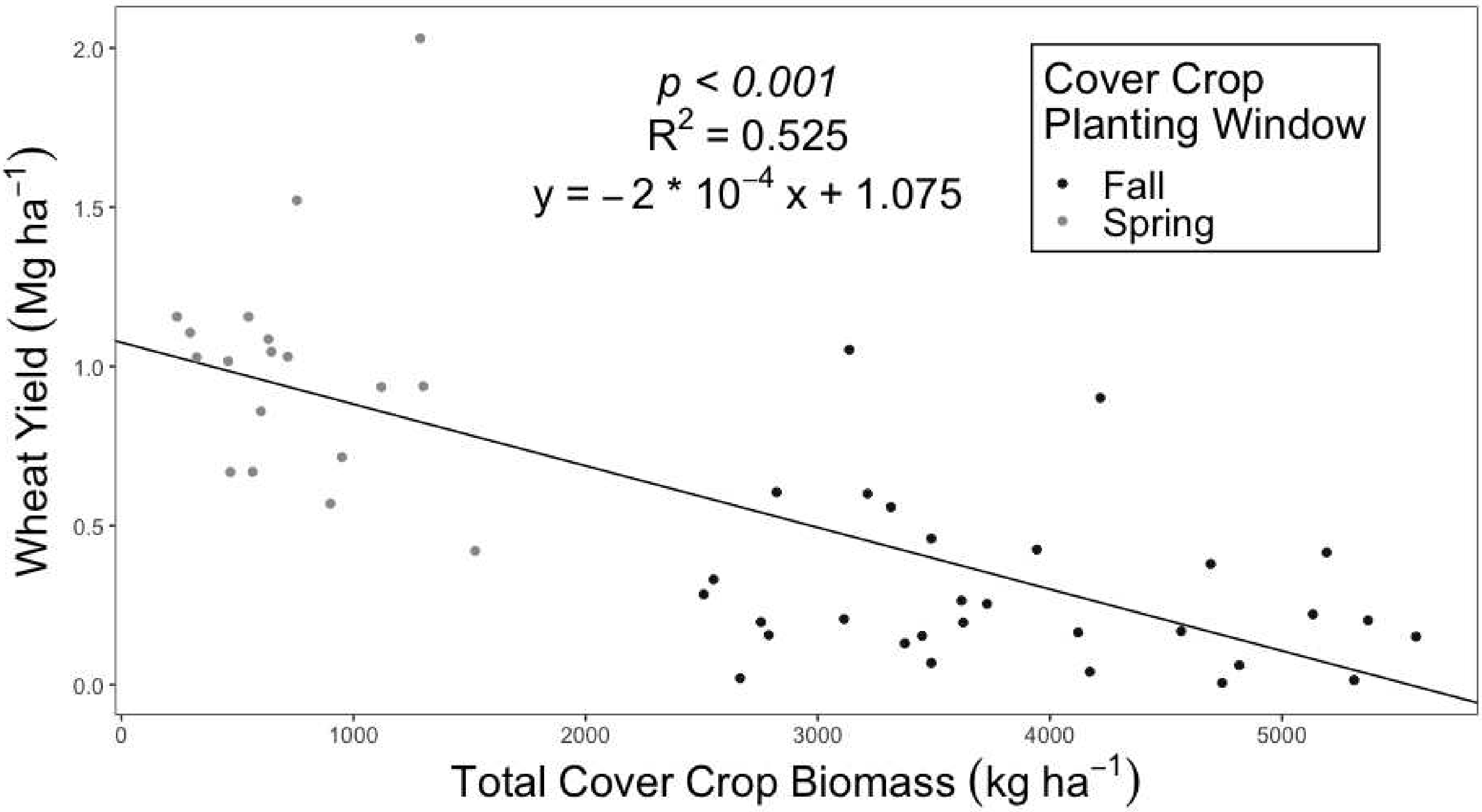

Figure 3.1. Correlation ($R^2 = 0.525$; $p < 0.001$) between fall- and spring-planted cover crop biomass (2017) and subsequent winter wheat yields (2018) in a field experiment (T2) located at the Southwestern Colorado Research Center near Yellow Jacket, Colorado.

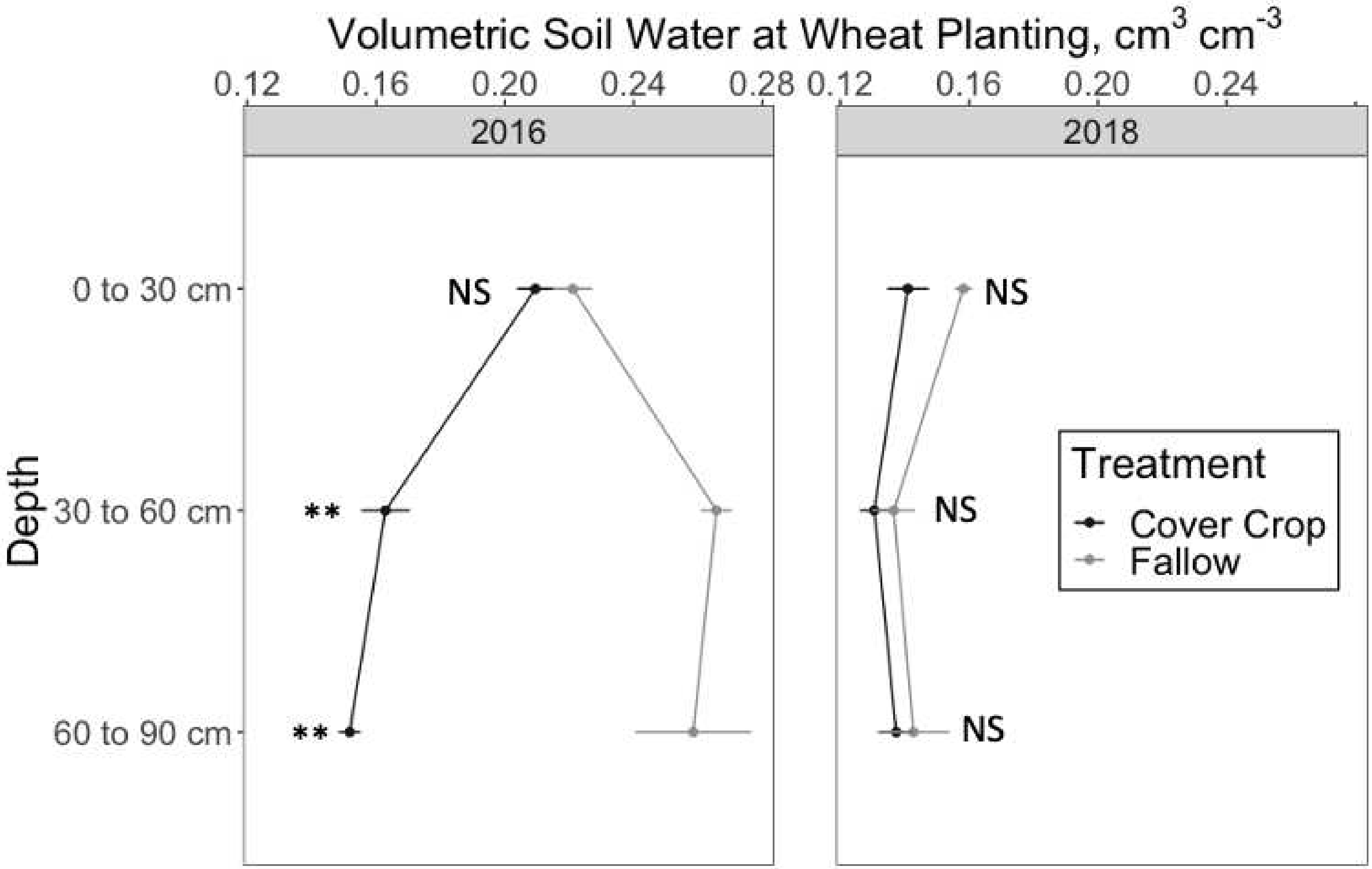

Figure 3.2. Volumetric soil water content at 2016 and 2018 wheat planting in a field experiment (T1) located at the Southwestern Colorado Research Center in Yellow Jacket, Colorado. Significance of orthogonal contrasts between cover crop and fallow treatments for each depth are displayed (NS means not significant, ** indicates $p < 0.01$).

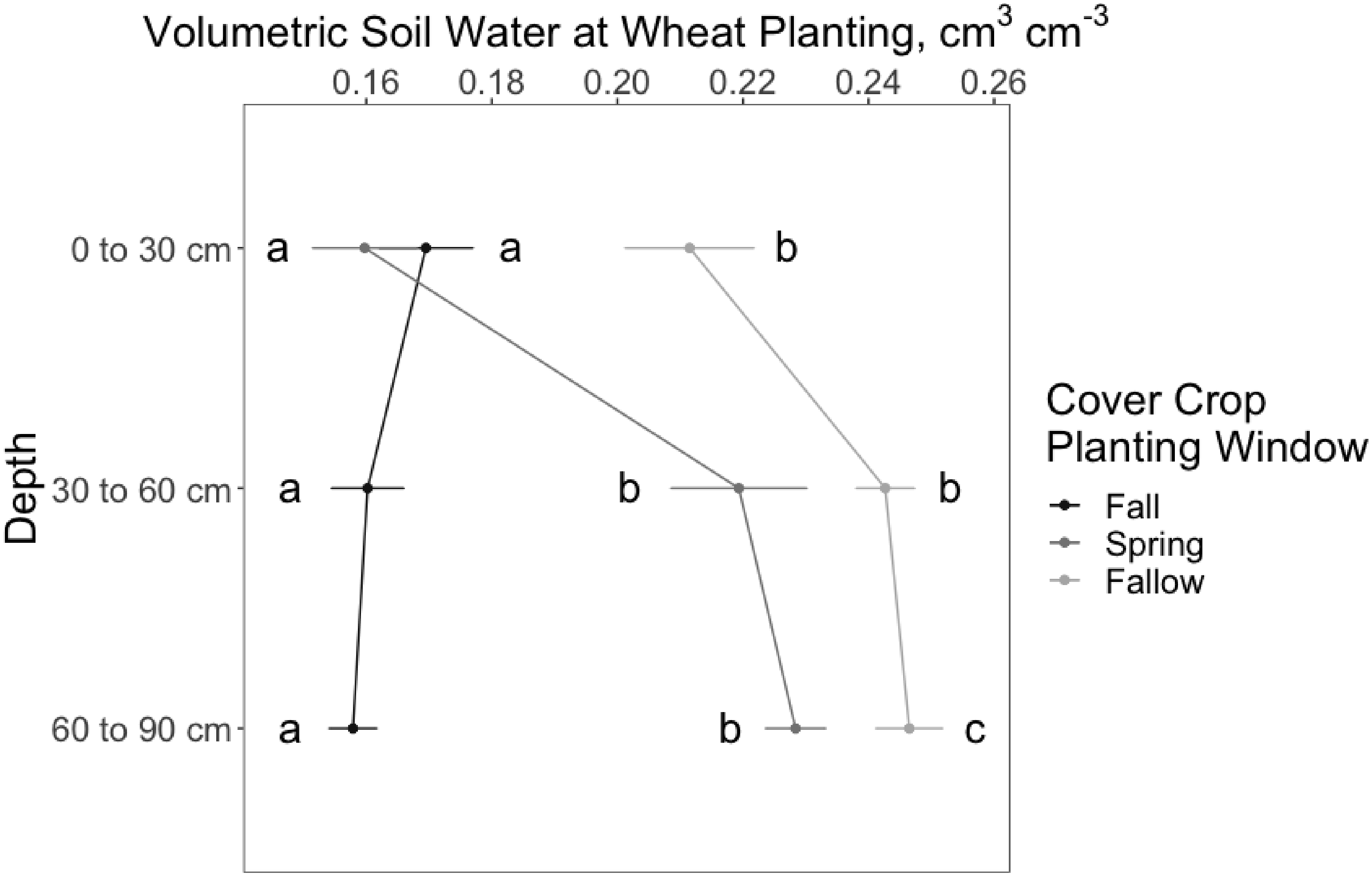

Figure 3.3. Soil water levels at 2017 wheat planting in a field experiment (T2) located at the Southwestern Colorado Research Center in Yellow Jacket, Colorado. Significance of orthogonal contrasts between spring- and fall-planted cover crop and fallow treatments for each depth are indicated by compact letter display, where groupings differ by an alpha level of 0.05.

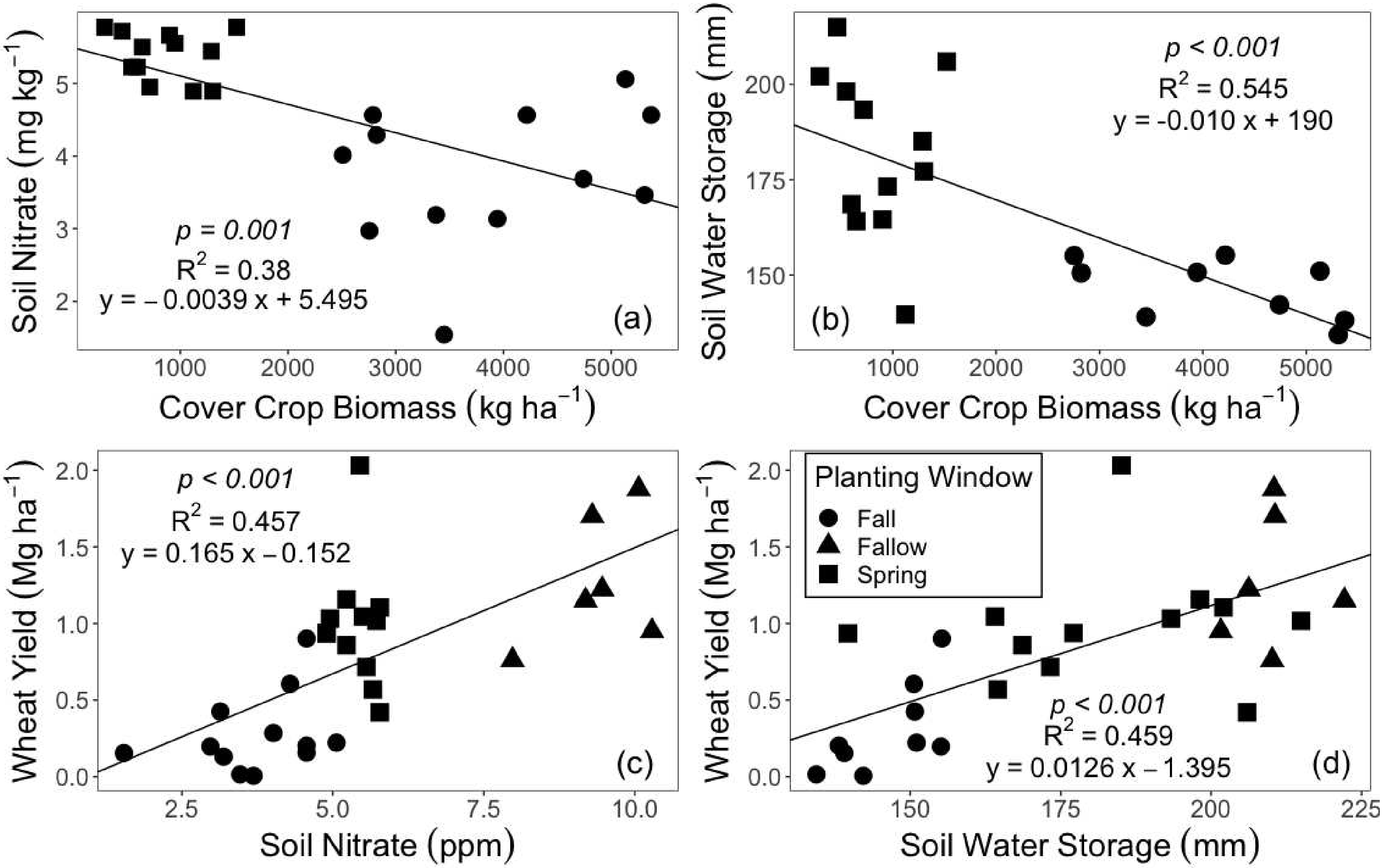

Figure 3.4. Correlations between 2017 cover crop biomass and subsequent (a) soil nitrate levels and (b) soil water storage in the top 90 cm of the soil profile at 2018 wheat planting in a field experiment (T2) located at the Southwestern Colorado Research Center in Yellow Jacket, Colorado. Correlations also shown between 2018 wheat yields and (c) soil nitrate levels and (d) soil water storage at wheat planting. Shapes indicate different cover crop planting windows. Multiple R^2, *p*-values, and equations for associated linear regressions are displayed on figures.

REFERENCES

Alewoye Getie, M., Legesse, S. A., Mekonnen, M., & Aschalew, A. (2020). Soil properties and crop productivity strategies as a potential climate variability adaptation option in Adefwuha Watershed, Ethiopia. *Earth Systems and Environment*, *4*(2), 359-368. https://doi.org/10.1007/s41748-020-00156-8.

Baraibar, B., Hunter, M. C., Schipanski, M. E., Hamilton, A., & Mortensen, D. A. (2018). Weed suppression in cover crop monocultures and mixtures. *Weed Science*, *66*(1), 121-133. https://doi.org/10.1017/wsc.2017.59.

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*(9), e0215702. https://doi.org/10.1371/journal.pone.0215702.

Berrada, A.F. (2012). *Southwestern Colorado Research Center 2012 Results (Technical Report)*. Colorado State University. Yellow Jacket, Colorado. https://mountainscholar.org/bitstream/handle/10217/80656/CAES_tr13-8.pdf?sequence=1&isAllowed=y

Blanco-Canqui, H., Stone, L. R., & Stahlman, P. W. (2010). Soil response to long-term cropping systems on an Argiustoll in the central Great Plains. *Soil Science Society of America Journal*, *74*(2), 602-611. https://doi.org/10.2136/sssaj2009.0214.

Burgess, M., Miller, P., Jones, C., & Bekkerman, A. (2014). Tillage of cover crops affects soil water, nitrogen, and wheat yield components. *Agronomy Journal*, *106*(4), 1497-1508. https://doi.org/10.2134/agronj14.0007.

Colorado Agricultural Statistics Service. (2018). *Colorado Agricultural Statistics, 2016–2017*; National Agricultural Statistics Service and Colorado Department of Agriculture: Lakewood, CO.

Colorado Agricultural Statistics Service. (2019). *Colorado Agricultural Statistics, 2017–2018*; National Agricultural Statistics Service and Colorado Department of Agriculture: Lakewood, CO.

Colorado Agricultural Statistics Service. (2020). *Colorado Agricultural Statistics, 2018–2019*; National Agricultural Statistics Service and Colorado Department of Agriculture: Lakewood, CO.

Finney, D. M., White, C. M., & Kaye, J. P. (2016). Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. *Agronomy Journal*, *108*(1), 39-52. https://doi.org/10.2134/agronj15.0182.

Finney, D. M., & Kaye, J. P. (2017). Functional diversity in cover crop polycultures increases multifunctionality of an agricultural system. *Journal of Applied Ecology*, *54*(2), 509-517. https://doi.org/10.1111/1365-2664.12765.

Florence, A. M., Higley, L. G., Drijber, R. A., Francis, C. A., & Lindquist, J. L. (2019). Cover crop mixture diversity, biomass productivity, weed suppression, and stability. *PLoS one*, *14*(3), e0206195. https://doi.org/10.1371/journal.pone.0206195.

Keeney, D. R., & Nelson, D. W. (1983). Nitrogen—inorganic forms. *Methods of soil analysis: Part 2 chemical and microbiological properties*, *9*, 643-698. https://doi.org/10.2134/agronmonogr9.2.2ed.c33.

Kelly, C., Schipanski, M. E., Tucker, A., Trujillo, W., Holman, J. D., Obour, A. K., ... & Fonte, S. J. (2021). Dryland cover crop soil health benefits are maintained with grazing in the US

High and Central Plains. *Agriculture, Ecosystems & Environment*, *313*, 107358. https://doi.org/10.1016/j.agee.2021.107358.

Kirkegaard, J. A., & Ryan, M. H. (2014). Magnitude and mechanisms of persistent crop sequence effects on wheat. *Field Crops Research*, *164*, 154-165. https://doi.org/10.1016/j.fcr.2014.05.005.

Kumar, V., Obour, A., Jha, P., Liu, R., Manuchehri, M. R., Dille, J. A., Holman, J. D., & Stahlman, P. W. (2020). Integrating cover crops for weed management in the semiarid US Great Plains: opportunities and challenges. *Weed Science*, *68*(4), 311-323. https://doi.org/10.1017/wsc.2020.29.

Helgason, B. L., Gregorich, E. G., Janzen, H. H., Ellert, B. H., Lorenz, N., & Dick, R. P. (2014). Long-term microbial retention of residue C is site-specific and depends on residue placement. *Soil Biology and Biochemistry*, *68*, 231-240. https://doi.org/10.1016/j.soilbio.2013.10.002.

Holman, J. D., Arnet, K., Dille, J., Maxwell, S., Obour, A., Roberts, T., Roozeboom, K., & Schlegel, A. (2018). Can cover or forage crops replace fallow in the semiarid Central Great Plains? *Crop Science*, *58*(2), 932-944. https://doi.org/10.2135/cropsci2017.05.0324.

Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2018). Emmeans: Estimated marginal means, aka least-squares means. *R package version*, *1*(1), 3.

Lyon, D. J., Nielsen, D. C., Felter, D. G., & Burgener, P. A. (2007). Choice of summer fallow replacement crops impacts subsequent winter wheat. *Agronomy Journal*, *99*(2), 578-584. https://doi.org/10.2134/agronj2006.0287.

Miguez, F. E., Archontoulis, S, & Basche, A. D. (2016). *Predicting Long-Term Cover Crop Impacts on Soil Quality Using a Cropping Systems Model (2016 Project Summaries)*;

Leopold Center for Sustainable Agriculture, Iowa State University. Ames, IA. http://lib.dr.iastate.edu/leopold_grantreports/504

Miller, P. R., Engel, R. E., & Holmes, J. A. (2006). Cropping sequence effect of pea and pea management on spring wheat in the northern Great Plains. *Agronomy Journal*, *98*(6), 1610-1619. https://doi.org/10.2134/agronj2005.0302.

Natural Resources Conservation Service. (2015). *National Resources Inventory (Summary Report)*; Natural Resources Conservation Service and Center for Survey Statistics and Methodology: Washington, DC.

Nielsen, D. C., Anderson, R. L., Bowman, R. A., Aiken, R. M., Vigil, M. F., & Benjamin, J. G. (1999). Winter wheat and proso millet yield reduction due to sunflower in rotation. *Journal of production agriculture*, *12*(2), 193-197. https://doi.org/10.2134/jpa1999.0193.

Nielsen, D. C., Vigil, M. F., Anderson, R. L., Bowman, R. A., Benjamin, J. G., & Halvorson, A. D. (2002). Cropping system influence on planting water content and yield of winter wheat. *Agronomy Journal*, *94*(5), 962-967. https://doi.org/10.2134/agronj2002.9620.

Nielsen, D. C., & Vigil, M. F. (2005). Legume green fallow effect on soil water content at wheat planting and wheat yield. *Agronomy Journal*, *97*(3), 684-689. https://doi.org/10.2134/agronj2004.0071.

Nielsen, D. C., Lyon, D. J., Hergert, G. W., Higgins, R. K., Calderón, F. J., & Vigil, M. F. (2015). Cover crop mixtures do not use water differently than single-species plantings. *Agronomy Journal*, *107*(3), 1025-1038. https://doi.org/10.2134/agronj14.0504.

Nielsen, D. C., Lyon, D. J., Higgins, R. K., Hergert, G. W., Holman, J. D., & Vigil, M. F. (2016). Cover crop effect on subsequent wheat yield in the central Great Plains. *Agronomy Journal*, *108*(1), 243-256. https://doi.org/10.2134/agronj2015.0372.

Nielsen, D. C., & Vigil, M. F. (2017). Intensifying a semi-arid dryland crop rotation by replacing fallow with pea. *Agricultural Water Management*, *186*, 127-138. https://doi.org/10.1016/j.agwat.2017.03.003.

R Core Team. (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Ranaivoson, L., Naudin, K., Ripoche, A., Affholder, F., Rabeharisoa, L., & Corbeels, M. (2017). Agro-ecological functions of crop residues under conservation agriculture. A review. *Agronomy for sustainable development*, *37*(4), 1-17. https://doi.org/10.1007/s13593-017-0432-z

Ruis, S. J., & Blanco-Canqui, H. (2017). Cover crops could offset crop residue removal effects on soil carbon and other properties: A review. *Agronomy Journal*, *109*(5), 1785-1805. https://doi.org/10.2134/agronj2016.12.0735.

Schipanski, M. E., Barbercheck, M., Douglas, M. R., Finney, D. M., Haider, K., Kaye, J. P., Kemanian, A. R., Mortensen, D. A., Ryan, M. R., Tooker, J., & White, C. (2014). A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agricultural Systems*, *125*, 12-22. https://doi.org/10.1016/j.agsy.2013.11.004.

Schlegel, A. J., & Havlin, J. L. (1997). Green fallow for the central Great Plains. *Agronomy Journal*, *89*(5), 762-767. https://doi.org/10.2134/agronj1997.00021962008900050009x.

Schwinning, S., Belnap, J., Bowling, D. R., & Ehleringer, J. R. (2008). Sensitivity of the Colorado Plateau to change: climate, ecosystems, and society. *Ecology and Society*, *13*(2). https://www.jstor.org/stable/26268003

Seager, R., Ting, M., Held, I., Kushnir, Y., Lu, J., Vecchi, G., Harnik, N., Leetmaa, A., Lau, N. C., & Naik, N. (2007). Model projections of an imminent transition to a more arid climate in southwestern North America. *Science*, *316*(5828), 1181-1184. https://doi.org/10.1126/science.1139601.

Smika, D. E. (1970). Summer Fallow for Dryland Winter Wheat in the Semiarid Great Plains 1. *Agronomy Journal*, *62*(1), 15-17. https://doi.org/10.2134/agronj1970.00021962006200010005x

Snapp, S. S., Swinton, S. M., Labarta, R., Mutch, D., Black, J. R., Leep, R., Nyiraneza, J., & O'neil, K. (2005). Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agronomy journal*, *97*(1), 322-332. https://doi.org/10.2134/agronj2005.0322a.

Soil Survey Staff. (2020). *Web Soil Survey*. U.S. Department of Agriculture, National Resources Conservation Service. Washington, DC.

Thomas, B. W., Larney, F. J., Chantigny, M. H., Goyer, C., & Hao, X. (2017). Fall rye reduced residual soil nitrate and dryland spring wheat grain yield. *Agronomy Journal*, *109*(2), 718-728. https://doi.org/10.2134/agronj2016.10.0616.

Thorup-Kristensen, K. (1993). The effect of nitrogen catch crops on the nitrogen nutrition of a succeeding crop: I. Effects through mineralization and pre-emptive competition. *Acta Agriculturae Scandinavica B-Plant Soil Sciences*, *43*(2), 74-81. https://doi.org/10.1007/BF00748941.

Tilman, D., Reich, P. B., Knops, J., Wedin, D., Mielke, T., & Lehman, C. (2001). Diversity and productivity in a long-term grassland experiment. *Science*, *294*(5543), 843-845. https://doi.org/10.1126/science.1060391.

Unger, P. W., & Vigil, M. F. (1998). Cover crop effects on soil water relationships. *Journal of soil and water conservation*, *53*(3), 200-207.

U.S. Geological Survey. (2002). *Precipitation History of the Colorado Plateau Region, 1900–2000 (Fact Sheet 119-02)*. U.S. Geological Survey. Flagstaff, AZ. https://pubs.usgs.gov/fs/2002/fs119-02/

CHAPTER 4: SYNERGIES AND TRADE-OFFS BETWEEN ECOSYSTEM SERVICES AND ECONOMICS IN DRYLAND COVER CROP SYSTEMS

4.1 Introduction

Prolonged bare fallow periods are often implemented in dryland systems to maintain stable crop production amidst water limitations (Peterson and Westfall, 2004). Despite improving yield stability, the fallow period is associated with harmful environmental consequences. Multiple tillage events are often used to control weeds, disrupting soil structure and leaving soil bare and susceptible to erosion (Liebig et al., 2004). Prolonged periods with no C inputs also deplete soil organic C (SOC) levels and associated soil nutrient reserves (Campbell et al., 2005). Over time, tilling and fallowing can lead to productivity declines due to inefficient soil water storage, loss of soil organic matter, poor soil structure, and high rates of soil erosion (Peterson et al., 2020).

On the semi-arid Colorado Plateau, dryland farmers cope with particularly low annual precipitation (less than 400 mm) by alternating winter wheat (*Triticum aestivum* L.) with 14-month bare fallow periods. The two-year winter wheat-fallow rotation has historically been preferred to continuous (summer) cropping, which can have higher annualized yields but increased crop failure risk (Zook, 1950; Nielsen and Vigil, 2018). Resulting soil degradation and erosion threatens the longevity of production in the region, leading dryland producers to explore alternative cropping systems and management strategies.

With the advent of glyphosate, some producers have switched to no-till management to address environmental and productivity concerns. While wheat-fallow systems managed under no-till experience reduced rates of SOC loss and erosion and slightly higher precipitation-use-efficiency, the extended fallow period limits the potential of no-till to fully address soil degradation. Precipitation-use-efficiency in no-till fallow periods is at most 40% (Peterson et al., 1996), and no-till wheat-fallow systems still result in gradual SOC and fertility decline (Ortega et al., 2002).

While no-till alone cannot reverse the detrimental impacts of conventional farming practices, benefits of precipitation storage in no-till systems have enabled cropping system intensification beyond the two-year wheat-fallow system in the semi-arid western U.S. Continuous cropping allows crops to take advantage of improved water storage by providing crop cover during late fall and winter periods, which are important periods for soil moisture storage due to higher rainfall levels and lower evapotranspiration rates (Peterson et al., 2020). A long-term study by Peterson et al. (1993) showed that continuous cropping in no-till systems resulted in 90% more aboveground biomass production than a 2-yr wheat-fallow rotation. In addition to productivity benefits, increased C input during otherwise fallow periods is critical for improving soil structure, infiltration rates, and SOC content (Shaver et al., 2002; Liebig et al., 2004).

Intensifying crop rotations through the integration of cover crops has been shown to support a suite of ecosystem services (ES) that can address the soil degradation and erosion that threaten dryland farming on the Colorado Plateau. ES are benefits that humans obtain from the environment and can be categorized into provisioning services, such as the production of food and fiber, supporting services, which support nutrient and water cycling, and regulating services, which provide environmental benefits external to the farm (Millennium Ecosystem Assessment, 2005). ES associated with cover crops include SOC accrual, N retention, erosion control, weed suppression, increased microbial activity, among others (Snapp et al., 2005; Daryanto et al., 2018). These ES potentially contribute to sustainable production and decreased input costs on-farm, and also provide benefits to society as a whole, such as reduced greenhouse gas emissions, improved air and water quality, and reduced erosion.

Despite these benefits, cover crop adoption rates remain low at only 5.1% nationally (Wallander et al., 2021). Low adoption rates may be attributed to productivity and economic trade-offs of cover cropping, due to increased production costs, potential competition with cash crops for water and nutrients, and the risk of untimely cover crop termination and cash crop planting (Snapp et al., 2005; Schipanski et al., 2014). Conservation incentives which compensate for these trade-offs are increasingly prevalent; in 2018, the U.S. Department of Agriculture spent more than 155 million USD in cover crop funding (Wallander et al., 2021) and launched a new Cover Crop Initiative in 2022 to expand cover crop incentives in 11 states, including Colorado (Natural Resources Conservation Service, 2022a). In addition to conservation incentives, grazing or haying cover crops has been put forth as a potential way to increase the economic viability of cover cropping (Saseendran et al., 2013; Holman et al., 2018).

Productivity trade-offs are particularly pronounced in dryland systems, where water availability largely drives crop yield. Replacing the fallow period with cover crops has been shown to reduce soil moisture at subsequent cash crop planting, resulting in significant yield penalties (Unger and Vigil, 1998; Nielsen et al., 2016a; Holman et al., 2018). Despite these findings, the benefits and trade-offs of cover crops have not been fully quantified in dryland systems, and the economic impact of cover crops remains uncertain. Furthermore, the Colorado Plateau is characterized by lower annual precipitation (less than 400 mm) and higher elevations (2000 m.a.s.l and above) than the Central and Northern Great Plains where the majority of the referenced dryland cropping systems research has been conducted. More research is needed to understand the potential for cover crops to address the challenges of soil degradation and erosion, quantify potential productivity and economic trade-offs, and identify management strategies that increase the viability of cover cropping in these conditions.

Our research sought to address this need by examining the benefits and trade-offs of cover cropping in dryland wheat systems on the Colorado Plateau. Using an interdisciplinary approach, we quantified key ES while also assessing economic impacts, as these determine feasibility of adoption. Economic impacts were quantified with and without the hypothetical sale of cover crop biomass as forage. We evaluated whether no-till management indeed allows for increased cropping intensification amidst water limitations by alleviating potential productivity trade-offs. We also assessed impacts of cover crop planting window (fall vs. spring), as we hypothesized that the presence of cover crops during late fall and winter would aid in precipitation-use-efficiency and erosion control in this critical period.

4.2 Methods

4.2.1 Experimental design

Two field trials were established side-by-side at the Colorado State University Southwestern Colorado Research Center near Yellow Jacket, Colorado (37°32′ N latitude; 108°44′ W longitude; 2100 m.a.s.l.). Precipitation in the region is bimodal, occurring mostly during the winter and in late summer, with an average of around 370 mm yr^{-1} (yearly average from 1991-2020; PRISM Climate Group, 2014). Average monthly high temperatures range from 3 °C in January to 31 °C in July. The field trials were established on relatively flat, homogenous land with Wetherill loam soil (fine-silty, mesic Aridic Haplustalfs; 36% sand, 41% silt, and 22% clay) (Soil Survey Staff, 2020). Baseline soils had low organic matter content (0.81% SOC) and neutral pH of 6.9. Prior to the establishment of trials, fields were in dryland, conventionally tilled production since 2010. Additional details regarding field history can be found in Eash et al. (2021), which presented the short-term productivity results of these trials.

In 2015, the first field trial (T1) was established to compare three fall-planted cover crop mixtures in rotation with winter wheat, with a fallow-winter wheat rotation as a control. The control treatment was based on the most common local practice in the region, which involves 14 months of bare fallow to allow for soil moisture recharge between wheat harvest (July) and planting (September). This trial was under no-till management, and weeds in the fallow treatment were controlled using herbicides (additional detail below). T1 followed a randomized complete block design with three replicate blocks. Plots in T1 were 6 m wide x 61 m long, or 372 m^2 each.

The second field trial (T2) was established in 2016 adjacent to T1. This trial similarly compared cover crop-winter wheat rotations against a fallow-winter wheat control, but also sought to evaluate effects of tillage regime (no-till vs. conventional tillage) and cover crop planting window, with five fall-planted mixtures and three spring-planted mixtures in a full-factorial design for a total of 18 treatments. T2 followed a split-plot design, with the two tillage treatments randomly allocated to three replicate blocks, while the nine cover crop treatments were randomly allocated to subplots (3.7 m x 30.5 m, or 113 m^2) within each tillage treatment. Along with allowing for the evaluation of cover crop management factors (tillage and planting window) on ES, the addition of T2 ensured that both crop phases of the 2-year rotation were represented each year, thus facilitating a better understanding of interannual variability at the field site.

Cover crop mixtures for both trials were developed together with local producers and other stakeholders and are presented in Table 4.1. The target seeding rate for all mixtures was 34 kg ha^{-1}, with slight variation among years and treatments. The rationale behind the selection of mixtures and seeding rates is detailed in Eash et al. (2021).

Planting and harvest dates for the two-year rotations in both trials followed local management norms (Table S4.1). Winter wheat (hard red winter wheat variety ‘Fairview’) was generally planted in September and harvested in July of the following year. In fall-planted cover crop treatments, mixtures were typically planted in September (1-2 months after wheat harvest) and terminated in June. In spring-planted cover crop treatments, mixtures were planted the following April or May and terminated in June or July. Control treatments remained fallow for 14 months, from wheat harvest in July until the next winter wheat planting (September). In accordance with local practices, wheat (hard red variety ‘Fairview’) was planted at a seeding rate of 56 kg ha^{-1} with rows spaced approximately 21 cm, and no fertilizer was added to either trial during the study.

Weed control and cover crop termination were accomplished through herbicide application in all no-till plots using a mixture of glyphosate (*N*-(phosphonomethyl)glycine) and 2,4-D amine (2,4-dichlorophenoxyacetic acid) and mechanically, using a tandem disk or field cultivator, in the tilled plots.

We measured the effects of cover crop mixture, planting window and tillage treatments on nine ES that were classified into groups (Table 4.2) defined by the Millennium Ecosystem Assessment (2005).

4.2.2 Wheat yield and yield stability

Wheat was harvested in July of each year from subplots in the center of each plot to avoid edge effects using a Hege plot combine (1.2 m width; 6 rows). Wheat grain was then cleaned using an electric winnower, weighed, and tested for moisture and density. All wheat yields are reported at 11% moisture content. For each treatment, yield stability was calculated as:

$$Yield\ Stability = 1 - \frac{standard\ deviation}{mean}$$

which included three cropping cycles for T1 and two cropping cycles for T2. Only two cropping cycles were included in T2 because the project ended before the third wheat harvest.

4.2.3 Forage production and quality

Forage production was estimated to be 50% of cover crop biomass. Cover crop biomass was measured at cover crop termination (Table S4.1) using a 0.25 m^2 quadrat with three subsamples randomly distributed per plot. Biomass was carefully cut at the soil surface (~1 cm height) and the three subsamples were combined. Biomass was then dried at 60°C and weighed.

Dried cover crop biomass from all treatments was finely ground and analyzed for forage quality using near-infrared reflectance spectroscopy. Relative forage quality (RFQ) scores were then calculated as:

$$RFQ = \frac{TDN * DMI}{1.23};$$

$$TDN = (NFC * 0.98) + (CP * 0.87) + (EE * 0.97 * 2.25) + (NDFn * NDFDp) - 10);$$

$$DMI = [-2.318 + (0.442 * CP) - (0.01 * CP^2) - (0.638 * TDN) + (0.000922 * TDN^2) + (0.18 * ADF) - (0.00196 * ADF^2) - (0.00529 * CP * ADF)$$

where TDN is total digestible nutrients, DMI is dry matter intake, NFC is non-fiber carbohydrates, CP is crude protein, EE is lipid soluble compounds, NDFn is nitrogen-free neutral detergent fiber, NDFDp is in-vitro NDF digestibility, ADF is acid digestible fiber. RFQ is an updated measure of relative forage value (RFV) that better predicts forage production potential (Jeranyama & Garcia, 2004). RFQ is calculated from TDN rather than digestible dry matter and accounts for variations in digestibility of the neutral detergent fiber fraction.

4.2.4 Soil sampling

Approximately one week prior to wheat planting, soils were sampled to a depth of at least 90 cm in 30 cm increments using a tractor-mounted Giddings probe (5 cm diameter). In 2016 and

2017, only one subsample per plot was collected, but starting in 2018, two subsamples were collected and combined for analysis. The target sampling depth was 180 cm, but the probe could not always penetrate to this depth due to dry conditions in the sub-surface soils. A 50 g subsample of each depth increment was weighed, dried at 105°C and reweighed to determine gravimetric moisture content. Gravimetric moisture content was then converted to volumetric moisture content by multiplying by the bulk density that corresponded to the sampling depth (1.35 g cm^{-3} for the 0-30 cm depth, 1.40 g cm^{-3} for the 30-60 cm depth, and 1.45 g cm^{-3} for the 60-90 cm depth; based on unpublished data from the study site). The remainder of the sample from the 0-30 cm depth increment was air-dried and a subsample was sent to Ward Laboratories in Kearney, NE for nitrate analysis using a flow injection analyzer (Keeney and Nelson, 1982).

To assess wet aggregate stability, soil was sampled to a depth of 5 cm at two sampling points per plot at termination of the third cover cropping cycle (2020 in T1 and 2021 in T2). Subsamples were combined, gently passed through an 8 mm sieve, and air-dried to further analyze aggregate stability by wet sieving, following methods adapted from Elliott (1986). In summary, a 40 g sample was placed on a 2 mm sieve and submerged in deionized water for 5 minutes for slaking. The sieve was then oscillated up and down (in and out of the water) 50 times during a 2 min period. Soil passing through the sieve was subsequently transferred to a 250 μm, and a 53 μm sieve. Soils remaining on each sieve were rinsed into a pan and were dried at 60 °C. The proportions of whole soil in each aggregate size fraction and average diameter for each fraction were used to calculate mean weight diameter according to van Bavel (1950).

Also at termination of the third cover cropping cycle, two subsamples per plot were taken to a depth of 15 cm using a soil probe (2 cm diameter). Using sterilized sampling equipment, soils from the center of the core were extracted and frozen for DNA extraction. The remainder of the subsamples were combined, air-dried, and passed through a 2 mm sieve. A subsample of this soil was then finely ground and analyzed for total C content using a LECO combustion analyzer (LECO Tru-SPEC, St. Joseph, MI).

4.2.5 Prokaryotic diversity and richness

Soils that were previously sampled to a depth of 15 cm at cover crop termination and frozen were processed for 16S rRNA amplicon sequencing using an Illuma Miseq platform. Soil DNA was extracted from 0.50 g of samples using the Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to manufacturer instructions. To characterize prokaryotic diversity and community composition we used the primer sets 515F/806R (Caporaso et al., 2012) that amplifies a portion of the bacterial 16S rRNA gene. A combination of USEARCH (Edgar, 2010) and UNOISE3 (Edgar, 2016) was used for bioinformatics processing. The USEARCH pipeline was used to generate Amplicon Sequence Variant (ASV) tables based on 97% sequence similarity. Sequencing run quality was assessed using fastQC (Andrews, 2010), and raw sequences were discarded if they were short in length (<100 bp), had a low quality score ($Q < 20$), or contained ambiguous nucleotides. Adapters and primers were removed using cutadapt (Martin, 2011). To demultiplex samples, paired-end reads were merged, and an initial quality check test was performed. The representative set database was created using the UCLUST and UPARSE algorithm (Edgar, 2013). Unique sequences were located and identified as unique ASVs, which were then clustered using DADA2 and DeNoised using uNoise3 (Xiong et al., 2021a, 2021b). ASV tables at the sample level were generated by mapping reads to the Silva database (Pruesse et al., 2007). Bacterial sequences that match host mitochondria and chloroplast were removed. ASV abundance tables were rarefied to the median count per sample to ensure relatively equal sampling depth. Alpha diversity (Shannon index) and species richness were calculated using the phyloseq package in R (McMurdie & Holmes, 2013).

4.2.6 Erosion control

Erosion (yearly soil loss) was estimated for all treatments using the Revised Universal Soil Loss Equation (RUSLE2) for water erosion and Wind Erosion Prediction System (WEPS) for wind erosion, both available through the Natural Resources Conservation Service (Widman, 2004; Agricultural Research Service, 2020). We ran the simulation on a Wetherill loam, with data populated from the Soil Survey Geographic (SSURGO) database with low organic matter and an average slope of 2%. Modelling was based on historical average daily weather data for Dove Creek, CO (NOAA). The model was run based on a custom management plan and crop rotation that reflected all herbicide, tillage, planting, and harvest events for each treatment over the six-year study period. Yearly average cover crop biomass data were included in the simulation for each tillage and cover crop treatment. Soil loss estimates from wind and water erosion were summed together for total estimated soil loss.

4.2.7 Economic analysis

The cover crop-wheat and fallow-wheat cropping cycles spanned 2-year periods and Net Present Value (NPV) methods were used to assess returns to investments in the cover crop both with and without the sale of 50% of cover crop biomass as forage. First, enterprise budgets were developed to determine the annual net returns (in USD ha^{-1} $cycle^{-1}$) for each tillage and planting window combination in each experiment. Gross revenues included wheat revenue as the primary cash crop. Revenue from the sale of cover crop biomass as forage was included as appropriate to compare net returns with and without forage sales. Wheat revenues were calculated using average annual wheat yields from the trials and wheat grain prices from Colorado State University (CSU)

Extension Crop Enterprise Budgets for dryland wheat production in Western Colorado (Colorado State University Extension, 2016; Colorado State University Extension, 2018; Colorado State University Extension, 2021). Operating expenses included seed costs (wheat seed and cover crop seed), chemical costs (herbicides), variable machinery costs (fuel, oil, repairs, labor) for field operations, and six months of interest expenses on operating capital. Inputs were assigned the same prices as the actual inputs used in the experiments and interest expenses were estimated using an interest rate of 7.5% based on the CSU Extension enterprise budgets. Ownership expenses (depreciation, interest, taxes, insurance, housing) were included for tillage, planting, weed control, harvest, and hauling field operations for the wheat cash crop and for planting and termination field operations for the cover crop. The operating and ownership expenses for machinery and equipment are from annual reports published by Iowa State University (Plastina, 2016; Plastina, 2018; Plastina, 2020). Other economic factors of production (e.g., overhead, land, unpaid labor and management) were assumed to remain constant across treatments and are therefore not included in the economic analysis.

Second, the equation used to determine the NPV for a given cropping cycle was:

$$NPV = \sum_{t=0}^{t=1} \frac{R_t - C_t}{(1+r)^t}$$

where t is year (either 0 or 1 in a 2-year cropping cycle), R_t is revenue gained in time period t, C_t are costs incurred in time period t, and r is the discount rate. We use the interest rate of 7.5% from the CSU Extension budget as the discount factor because it is a short-term financial analysis. Prior to discounting, revenues and costs were adjusted for inflation using the producer price index (U.S. Bureau of Labor Statistics, 2022). The average NPV per cycle was obtained for each treatment and trial combination by dividing total NPV over the three cycles. Changes in net returns for each combination of cover crop planting window and tillage treatment were calculated using the net returns of fallow and corresponding tillage treatment as reference treatments. Positive values therefore represent an increase in net returns and negative values indicating a decrease in net returns due to cover crops as compared to the reference treatments.

When including revenue from forage sales, we estimated forage yields to be 50% of total cover crop biomass production to ensure a level of soil cover consistent with cover crop best management practices (Natural Resources Conservation Service, 2021). Yields were assumed to contain 15% moisture and priced as hay on a USD ton^{-1} basis (Ponderosa Partnership, 2018). Hay prices were based on relative forage quality scores calculated for each cover crop treatment from the experiment and reported grass hay prices from the Agricultural Market Service (Agricultural Marketing Service, 2017; Agricultural Marketing Service, 2019; Agricultural Marketing Service, 2021). All RFQ values were in Grade 1 or 2 quality standard ranges [on the American Forage and Grassland Council scale of 1 to 5 (1 being best; Marsalis et al., 2009)] and prices corresponded to "Good" quality grass hay in the Agricultural Market Service reports (Agricultural Marketing Service, 2017; Agricultural Marketing Service, 2019; Agricultural Marketing Service, 2021). To verify that the prices were reasonable for the study area, local hay farmers corroborated similar hay prices during the years of study. Additional operating and ownership expenses for raking, mowing, and baling were included and are also sourced from annual reports published by Iowa State University (Plastina, 2016; Plastina, 2018; Plastina, 2020). A summary of the expense and revenue data by treatment is found in Table S4.2.

4.2.8 Statistical analysis

Individual ES indicators were analyzed using a multifactor ANOVA in the *lme4* package in R statistical software (Bates et al., 2015; R Core Team, 2021). For each response variable, trials and cropping cycles were analyzed separately. The exceptions to this were erosion estimates, which were not statistically analyzed, and yield stability, which integrated all cropping cycles. In T1, cover crop planting window was included as a fixed effect and block was included as a random effect. In T2, cover crop planting window, tillage and their interaction were included as fixed effects and block and block x tillage subplots were included as random effects. Normality and homogeneity of variance were verified, and no transformations were required. Tukey-adjusted pairwise comparisons were generated by the *emmeans* package in R to estimate differences among treatments (Lenth et al., 2022).

A linear mixed effect model was used to determine the relationship between wheat yield penalty, cover crop biomass production, and growing season precipitation. Cover crop biomass production in the previous year, precipitation received during wheat growth (August to July), and their interaction were included as continuous predictors and year, field, and block were included as random effects.

Our analyses focused on cover crop planting windows instead of cover crop mixture effects because, despite planting different mixtures, stand expression was very similar among cover crop treatments of the same planting window. This is due to extremely dry conditions that caused only a few species to dominate (namely grasses and winter pea). To confirm that cover crop mixture did not affect biomass production or other response variables, multifactor ANOVA was also performed using methods described above, replacing cover crop planting window with cover crop mixture as a main effect (findings reported in Table S4.3, S4.4).

To visualize treatment effects on multiple ES, ES proxies were converted to a standardized unit ranging from 0.1 to 1 using the following homothetic transformation equation:

$$Y_i = 0.1 + \left(\frac{x_i - b_i}{a_i - b_i}\right)$$

where Y is the transformed value of the ES i, x is the original indicator value, and a and b are the maximum and minimum observed values of the ES i, respectively (Kearney et al., 2019). A standardized value for belowground biodiversity was calculated for each plot by averaging together values for prokaryotic alpha diversity and richness. The transformed value (Y) of soil loss was subtracted from 1.1 to set all variables on a 'more-is-better' scale for erosion control. ES indices for soil water availability, N supply, food production and biomass production were calculated from averages across all cycles. Radar plots were generated to visualize trade-offs among ES. We note that this analysis was only performed for T2 to ensure a more robust analysis considering both tillage regime and cover crop planting window effects.

Bundling and trade-offs among individual ES were assessed by performing principal components analysis (PCA) on transformed ES values. To evaluate tradeoffs among ES categories (provisioning, supporting, and regulating), transformed values of ES were averaged within each category with each ES weighted equally.

4.3 Results

4.3.1 Provisioning services

When averaged across all years and both trials, wheat yields following fall-planted cover crops were 48% lower than those in fallow control plots (0.77 Mg ha^{-1} in fall-planted cover crop plots vs. 1.6 Mg ha^{-1} in fallow plots). However, this yield penalty was not consistently observed in all years and varied considerably in magnitude from year to year (Fig. 4.1). Spring-planted cover crops presented less of a productivity trade-off, as the average wheat yield penalty was just 24% (Table 4.3, 4.4). In T2, wheat yields in no-till plots tended to be higher on average than in tilled plots, although the magnitude of the cover crop yield penalty was similar, and this effect was only marginally significant in Cycle 1 of T2 ($p = 0.059$; Table 4.3).

The wheat yield penalty depended on an interactive effect between cover crop biomass production in the previous year and precipitation received during the wheat growing season ($p = 0.023$; Fig. 4.2). Cover crop biomass and wheat yields had a negative correlation when growing season precipitation was particularly low. For example, when only 158 mm of precipitation is received during wheat growth, such as in Cycle 1 of T2, each 100 kg ha^{-1} of cover crop biomass produced reduced subsequent wheat yields by an estimated 1.3% (+/- 0.1%). Conversely, when growing season precipitation is closer to average (above 265 mm) there is no significant relationship between cover crop biomass and subsequent wheat yield ($p > 0.05$; Fig. 4.2). In Cycle 2 of T1, which received the most precipitation during wheat growth, cover cropped plots actually yielded 12% more than wheat grown following the bare fallow period ($p = 0.009$; Fig. 4.1; Table 4.3, 4.4).

Yield stability was also lower following cover crops in both T1 and T2 ($p = 0.002$ and $p < 0.001$, respectively). In T2, there was a significant interactive effect ($p < 0.001$) between tillage and cover crop window in which yield stability was significantly decreased by fall-planted cover crops in tilled plots but was similar to the fallow control in no-till plots (Table 4.4).

Potential forage production was highly variable among years, ranging from a yearly average of 605 to 2560 kg ha^{-1} in fall-planted plots and from 370 to 1040 kg ha^{-1} in spring-planted plots. As potential forage was estimated to be 50% of cover crop biomass, cover crop biomass production was double these estimates (1210 to 5020 kg ha^{-1} in fall-planted plots and 740 to 2080 kg ha^{-1} in spring-planted plots; Table 4.3). Tillage regime only had a significant impact on cover crop biomass in Cycle 3 of T2, when spring-planted cover crops in no-till plots produced significantly more biomass than in tilled plots ($p < 0.001$ for planting window x tillage interactive effect).

4.3.2 Supporting services

Soil water storage at wheat planting was generally lower in fall-planted cover crop plots as compared to fallow control plots (Table 4.3). The exception to this was in Cycle 2 of T1, when little cover crop biomass was produced, and soil water caught up to fallow levels between cover crop termination and wheat planting. Following spring-planted cover crop plots, this difference was much less pronounced (Table 4.3). Tillage did not significantly affect soil moisture at wheat planting in any year.

A parallel trend was observed for soil nitrate at wheat planting, though the difference between cover cropped and fallow control plots decreased and became less significant throughout the course of the study. In T1, the difference between cover cropped and fallow plots decreased from 7.17 mg kg^{-1} ($p < 0.001$) in Cycle 1 to 2.29 mg kg^{-1} ($p = 0.04$) in Cycle 3. In T2, there was no significant difference in soil nitrate at wheat planting by Cycle 3 ($p = 0.13$; Table 4.3).

After three cover crop cycles, there was a significant effect on soil structure, as fall-planted cover crop plots had greater aggregate stability than fallow control plots in both T1 and T2 ($p = 0.05$ and $p < 0.001$, respectively; Table 4.3). There was no significant effect on aggregation in spring-planted cover crop plots ($p > 0.05$; Table 4.3). No effects of cover crops or tillage were observed on belowground prokaryotic diversity as measured by both the Shannon Diversity Index and richness (Table 4.4).

4.3.3 Regulating services

While there were no differences in soil C stocks among treatments, fall-planted cover crops suggested an improvement in erosion control that was particularly pronounced in tilled plots. Overall, modelled annual soil loss was lower in fall-planted cover crop plots as compared to fallow control plots. Soil loss in tilled, fallow treatments was substantially higher than in no-till fallow plots (7289 and 568 kg ha^{-1} soil loss yr^{-1}, respectively; Table 4.4). Tilled, fall-planted cover crop plots experienced the same amount of annual soil loss as no-till, fallow plots. In both tillage regimes, spring-planted cover crop treatments were moderately effective in controlling erosion, but not to the extent of fall-planted cover crops.

4.3.4 Economic analysis

Before considering revenue from forage sales, both fall- and spring-planted cover crop treatments substantially decreased net returns as compared to the fallow treatment. Net returns in fallow plots were already quite low and extremely variable among cropping cycles, ranging from -57 USD ha^{-1} in tilled, fallow plots in Cycle 1 of T2 to 210 USD ha^{-1} in no-till fallow plots in Cycle 1 of T1 (Table 4.5). Fall-planted cover crops decreased net returns by 176 USD ha^{-1} cycle^{-1} when averaged across both trials due to additional costs and reduced wheat revenue (Table 4.6). Additional costs due to cover cropping (average 75 USD ha^{-1} cycle^{-1}) were similar regardless of tillage regime and represented 44% of the change in net returns in fall-planted plots. Average reduction in wheat revenue following fall-planted cover crops was 102 USD ha^{-1} cycle^{-1} in no-till plots and 84 USD ha^{-1} cycle^{-1} in tilled plots (Table 4.5). Though the reduction in wheat revenue was less pronounced following spring-planted cover crops, additional costs associated with weed control and planting caused the decrease in net returns to be only slightly less than in fall-planted plots (141 USD ha^{-1} cycle^{-1} and 97 USD ha^{-1} cycle^{-1} in no-till and tilled plots, respectively) with a greater proportion attributed to additional costs (73%; Table 4.5, 4.6).

With revenue from forage sales, cover cropping costs and revenue losses were more than offset in T2 for fall-planted cover crop treatments (Table 4.5, 4.6). Additional revenue from the sale of forage averaged 420 USD ha^{-1} cycle^{-1} in fall-planted cover crop plots, while extra costs from mowing, raking, and baling hay averaged only 87 USD ha^{-1} cycle^{-1} (Table 4.5). In T2, fall-planted cover crops increased net returns compared to the fallow treatment in both cycles by an average of 161 USD ha^{-1} $cycle^{-1}$ in no-till plots and 176 USD ha^{-1} $cycle^{-1}$ in tilled plots (Table 4.6). Two out of three cycles in T1 also experienced an increase in net returns due to fall-planted cover crops. However, Cycle 3 had low cover crop biomass production, a large wheat yield penalty, and increased wheat market prices, and net returns were decreased by 274 USD ha^{-1} $cycle^{-1}$ (Table 4.6). As spring-planted cover crops produced less biomass, forage sales had less potential to offset costs and revenue loss in these plots. Spring-planted cover crops reduced net returns by an average of 95 and 43 USD ha^{-1} $cycle^{-1}$ in no-till and tilled plots, respectively (Table 4.6).

4.3.5 Ecosystem service trade-offs

When ES indices were averaged by category, there was a significant interactive effect between cover crop treatment and tillage regime for provisioning services ($p = 0.0026$; Table 4.4). No-till plots for all cover crop treatments scored highest, owing to greater yield stability and trends of higher forage and wheat production. Fall-planted cover crop treatments in tilled plots scored lowest, with lower yield stability and lowest average wheat yield. The fallow treatment, particularly in no-till plots, scored significantly higher in supporting services ($p = 0.0014$; Table 4.4). Higher soil moisture and soil nitrate levels outweighed greater aggregation in fall-planted cover crop plots. Indices for regulating services were driven by soil loss, as no differences in SOC content were observed.

Principal components analysis shows trade-offs among individual services in T2. Net returns and ES associated with wheat productivity (wheat yield, yield stability, soil moisture and N supply) were bundled and showed a negative correlation with forage production, erosion control and soil structure (Fig. 4.3). These services are primarily aligned with the first principal component axis (PC1), which explains 60.5% of the variance in composite indices. Fall-planted cover crop plots, under both conventional and no-till management, were clustered on the right side of the plot, indicating higher scores in forage production, erosion control and soil structure. Fallow plots clustered to the left with ES associated with wheat productivity and economics. No-till and tilled fallow plots clustered separately on the left, presumably due to low erosion control scores in tilled, fallow plots and slightly higher scores for yields and soil water availability in no-till plots (Table 3,4). Spring-planted treatments were located in the middle of these two ecosystem bundles, with lower wheat productivity trade-offs than fall-planted cover crop plots and only moderate erosion control benefits (Table 4.3, 4.4; Fig. 4.3).

Radar plots display a summary of these ES trade-offs (Fig. 4.4). Three of the nine ES evaluated, namely erosion control, forage production and soil structure, increased in fall-planted cover crop plots, while four were greater in fallow plots. N supply and soil water availability were lowest in fall-planted cover crop plots and highest in fallow plots. These two factors likely led to lower wheat yield and net returns (without considering forage revenue) of fall-planted cover crops across both tillage regimes. Yield stability in cover cropped tilled plots was significantly lower than in fallow tilled plots. C storage and belowground biodiversity were unaffected by tillage and cover crop treatment during the study period.

4.4 Discussion

The integration of cover crops into dryland wheat systems on the Colorado Plateau showed both beneficial and unfavorable impacts on multiple ecosystem services and economic returns. The management decisions of tillage, cover crop planting window and the potential to utilize cover crop biomass for forage each impacted overall system outcomes. In general, the integration of fall-planted cover crops, the use of no-till management, and utilizing cover crops for forage provided the most potential for maximizing ES benefits while integrating cover crops in an economically viable manner.

4.4.1 Ecosystem service synergies and trade-offs

ES provided by cover crops were bundled into groups linked to cover crop biomass production and those linked to wheat productivity. Similar ES bundling has been shown in more humid climates (Finney et al., 2016), and our study confirms that these relationships also exist in water-limited systems. Fall-planted cover crops improved three of the nine ES evaluated, namely erosion control, forage production, and soil structure, which had a trade-off with services associated with wheat productivity (N supply, crop production, yield stability, and soil water availability) that were higher in fallow control plots (Fig. 4.3, 4.4). This trade-off suggests that cover crops can be effective at improving soil properties and mitigating soil loss in water-limited conditions such as on the Colorado Plateau, but productivity declines and economic trade-offs may limit their adoption.

The 14-month bare fallow period with mechanical weed control that is typical in dryland systems on the Colorado Plateau leaves the soil extremely susceptible to wind and water erosion (Fryrear, 1981; Peterson et al. 2020). Soil loss estimates in tilled, fallow plots were 7300 kg ha^{-1} yr^{-1}, far greater than the NRCS soil loss tolerance rate of 2500 kg ha^{-1} yr^{-1}, which is a threshold of erosion that is considered to result in little or no long-term degradation of the soil (Natural Resources Conservation Service, 2001). The integration of a fall-planted cover crop was effective at reducing soil loss by an estimated 92% to a level that is well below this threshold (Table 4.4). Fall-planted cover crops have been shown to reduce wind and water erosion by providing protective soil cover, anchoring soil through extensive root systems, and decreasing runoff (Kessavalou & Walters, 1999; Ghimire et al., 2018). Soil cover in winter and spring months can be particularly important for preventing water erosion, as it dampens the impact of raindrops on the soil surface and protects against erosion caused by the melting of accumulated snowpack (Dabney et al., 2001). In contrast, spring-planted cover crops did not offer protection during these months and resulted in limited erosion control, mostly due to lower wind erosion.

No-till management has been shown to lower erosion potential, even in traditional wheat-fallow systems (Smika, 1990; Hansen et al., 2012). Our soil loss estimates confirm this finding, as no-till fallow treatments had comparable estimated rates of soil loss to tilled, fall-planted cover crop plots, suggesting that erosion control benefits can be achieved without the use of cover crops. Within no-till treatments, the presence or absence of cover crops had a relatively small effect on erosion potential. However, in systems in which herbicides cannot be used for weed control, such as in organic production or in the presence of herbicide-resistant weeds, planting fall cover crops is an effective strategy to mitigate soil loss associated with tillage.

Soil structure as measured by wet aggregate stability was also improved following three cycles of fall-planted cover crops, but these benefits were not observed in spring-planted cover crop plots. Cover crops have been shown to improve aggregation even in short timeframes in dryland and humid environments alike (Blanco-Canqui & Ruis, 2020; Kelly et al., 2021). Living roots and additional biomass associated with cover crops can support SOC levels, microbial activity, and physical enmeshment of primary soil particles, which lead to the formation of aggregates (Bronick & Lal, 2002). Improved aggregate stability in turn has important implications for a number of ES, including erosion control, water capture, SOC stabilization, and reduced compaction (Barthes & Roose, 2002; Six & Paustian, 2014). Improved water capture (via infiltration) could be particularly important for this region, where late-summer precipitation is often characterized by high intensity, short duration rain events with potential to result in runoff

(Schwinning et al., 2008). Increased aggregation following fall-planted cover crops was only detected in no-till plots, suggesting that cover crops must be planted under no-till to maintain improvements in soil structure. It is important to note, however, that improvements in aggregation without increases in SOC have been found to be relatively short-lived following cover crop termination, so continued cover cropping may be necessary to maintain improvements in soil structure and associated benefits in infiltration, erosion control, among others (Blanco-Canqui et al., 2013; Garcia-Gonzalez et al., 2016).

Despite increased biomass production during what is typically a bare fallow period, there were no differences in soil C content in cover cropped plots relative to fallow control plots after three cover crop cycles. Cover crops are widely used as a strategy to increase soil organic matter and sequester C (Poeplau and Don, 2015; McClelland et al., 2021), but increases in SOC due to cover crops can be inconsistent and variable in water-limited regions. A study in dryland systems in Kansas showed similar levels of SOC in fallow and cover crop plots, which authors attributed to variability in cover crop residue inputs due to several years of drought (Simon et al., 2022). In the present study, cover crop biomass was similarly variable and followed by wheat yield penalties, which were quite significant in some years, so net C inputs may have been similar among treatments in some cycles. However, increases in SOC have also been observed following five cover crop cycles in dryland systems, suggesting that a longer study period might be required to observe differences among treatments (Blanco-Canqui et al., 2013). Furthermore, differences in aggregation can often be an early indicator of changes in SOC, as physical protection of organic matter within aggregates can increase persistence in the soil (Six and Paustian, 2014). Still, SOC increases due to cover crops do not appear to be a consistent or short-term outcome in water-limited regions.

The integration of CC into crop rotations has been linked to increases in microbial diversity, which can lead to improvements in nutrient cycling, C storage, and crop productivity (Venter et al., 2016; Maron et al., 2018; Fan et al., 2021). However, CC effects on microbial diversity can be slight and/or highly variable (Kim et al., 2020), and no changes were detected among treatments in the present study. As water availability in dryland systems is shown to drive microbial activity and abundance (Angel et al., 2013; Maestre et al., 2015), depleted soil moisture levels following CC may have limited CC improvements to the soil microbiome.

Cover crop biomass production, soil structure improvements and erosion control benefits were associated with significant wheat productivity and economic tradeoffs. Regulating and supporting ES can be negatively correlated with provisioning services (Wittwer et al., 2021), particularly in dryland environments, where the elimination of a fallow period presents competition with cash crops for water (Unger and Vigil, 1998; Nielsen et al., 2016a). Wheat yields and associated metrics, such as water availability at wheat planting, N supply, and yield stability, were generally lower following fall-planted cover crops. The average 48% reduction in wheat yields following fall-planted cover crops is comparable to values found in other dryland wheat systems in the U.S. Great Plains (Lyon et al., 2004; Nielsen and Vigil, 2005; Aiken et al., 2013).

Yield penalties due to cover crops are also extremely variable in dryland systems, which is consistent with findings that show that reduced fallow periods result in lower yield stability and greater risk of crop failure (Zook, 1950; Nielsen and Vigil, 2018). Fallow replacement in the current study resulted in lowered wheat yield stability (Table 4.4), representing an increased risk associated with cover cropping that could be a large barrier to adoption. Throughout the course of the study period, the wheat yield penalty following fall-planted cover crops ranged from an 80% decrease to a 12% increase (Fig. 4.1; Table 4.3). A table published by Nielsen et al. (2016a) summarized published dryland yield penalties due to cover crops and showed that they similarly ranged from a 79% reduction to a 5% increase. Interannual variability in yield penalties is due in part to variable cover crop biomass production and wheat growing-season precipitation (Fig. 4.2; Nielsen and Vigil, 2005).

Wheat yields throughout the study period were tightly correlated with soil moisture at wheat planting; each 1000 kg ha^{-1} of cover crop biomass produced resulted in a 10 mm decrease in soil moisture at wheat planting, and wheat yields in turn decreased by 130 kg ha^{-1} per 10 mm reduction in soil moisture at planting (Eash et al., 2021). This relationship confirms other findings that water availability is the primary limitation for wheat growth and driver of the yield penalty in dryland systems (Reese et al., 2014; Nielsen et al., 2016).

Soil nitrate levels were also lower in cover cropped plots but are less likely to be a driving cause of lowered wheat yields. There is evidence that grass-dominant cover crop mixtures (such as those tested here) may negatively impact cash crop yields due to decreased soil N availability, but this effect is primarily observed in crops with a high N demand and in systems in which moisture is not limiting (Finney et al., 2016; Hunter et al., 2019). In the present study, differences in soil nitrate levels tended to level off by the third cropping cycle, indicating that N immobilized in cover crop biomass had mineralized back into the soil, while wheat yield penalties remained significant (Table 4.3). Furthermore, a separate study in these plots showed that the application of foliar N in the early spring successfully increased wheat yields only in fallow plots, where moisture limitations were not as pronounced (*unpublished results)*.

An increase in wheat yields following fall-planted cover crops was observed in Cycle 2 of T1, which experienced average precipitation levels in the wheat growing season and low cover crop biomass production in the previous year (Table 4.3, Fig. 4.1). A study in dryland systems in the Northern Great Plains also showed a 5% increase in wheat yields following a pea cover crop relative to chemical fallow in a year with average rainfall (Miller et al., 2011). As soil moisture availability and soil N levels were similar among treatments at the time of wheat planting, yield increases could have been due to other benefits provided by cover crops, such as reduced evaporative losses due to soil residue cover, more timely release of N throughout the wheat growing cycle, or improved porosity that is favorable to root growth (Snapp et al., 2005).

As ES associated with wheat productivity (soil nitrate, soil water availability, yield stability) were bundled and negatively associated with ES linked to cover crop biomass (forage production, erosion control, soil structure), limiting biomass production may be a way to reduce productivity trade-offs. Finney et al. (2016) show that productivity trade-offs can be balanced by planting legume cover crops that produce less biomass and have lower C:N ratios, thereby producing more timely release of N and resulting in less depletion of soil moisture. Earlier termination or later planting dates can also reduce yield penalties (Unger and Vigil, 1998; Nielsen and Vigil, 2005). In the present study, spring-planted cover crops presented fewer trade-offs in terms of wheat productivity and associated metrics but did not offer the soil and erosion control benefits seen in fall-planted cover crop plots (Table 4.3, 4.4; Fig. 4.4).

No-till management is considered to be conducive to cropping system intensification in dryland systems, as improved precipitation storage can allow for a shortened or reduced fallow period (Hansen et al., 2012; Peterson et al., 2020). No-till treatments scored slightly higher in provisioning services (Table 4.4; Fig. 4.4) since yield stability did not significantly decrease in fall-planted cover crop plots and no-till plots yielded slightly higher in wheat and in cover crop biomass in some years (Table 4.3). However, significant differences in soil moisture at wheat planting between tillage regimes were not detected, likely since these measurements were taken at wheat planting after no-till plots had supported greater biomass production and therefore greater transpiration (i.e., Cycle 3 of T2; Table 4.3). Particularly in years with below-average precipitation, plant-available water was depleted by cover crop termination. Results show that no-till management could provide some insurance in the integration of cover crops, but more rigorous measurement surrounding precipitation-use-efficiency is required. Furthermore, no-till benefits are slow to accrue, particularly in dry environments, and the effects of no-till may be more pronounced after more study years (Blevins et al., 1983).

4.4.2 Economic Analysis

The ES benefits of cover cropping observed here were associated with a significant decrease in net returns when forage revenue was not considered. Regulating ES, like C storage and erosion control, often present trade-offs in farm economics (Pilgrim et al. 2010; Schipanski et al., 2014), and the use of cover crops to improve ES has been shown to have variable effects on profitability (Bergtold et al., 2019). A study in Illinois corn-soybean systems showed change in net returns due to cover crops of -264 USD ha^{-1}, also primarily due to reductions in cash crop yields, though in these systems N limitations were driving yield penalties rather than water (Thompson et al., 2020). Partial budget analysis in regions with more precipitation typically experience small or no yield penalty following cover crops and may experience lower changes in net returns. For example, a study in central Pennsylvania in soybean-wheat-corn systems showed an average reduction in profitability of -63.97 USD ha^{-1} yr^{-1} in cover cropped systems mostly due to direct costs (Schipanski et al., 2014). In the present study, ES provided by fall-planted cover crops were associated with an average decrease in net returns of 176 USD ha^{-1} cycle^{-1} compared to the fallow, with a smaller portion (44%) attributed to increased costs due to higher yield penalties following cover crops in dryland environments.

The variability in net returns associated with cover crops could represent a significant barrier to adoption. Variability and risk associated with net returns has been shown to have a significant influence on the adoption of conservation practices (Larson et al., 2001; Adusumilli et al., 2020). As the change in net returns was largely dependent on yield penalties, it reflected the high variability in wheat yields that was observed in fall-planted cover crop plots. The decrease in net returns compared to the fallow was as low as 58 USD ha^{-1} cycle^{-1}, when a slight increase in wheat revenue following cover crops partially offset direct costs. In Cycle 3 of T1, however, a sharp increase in wheat prices coupled with an 80% yield penalty contributed to the largest decrease in net returns observed in all cycles (274 USD ha^{-1} cycle^{-1}; Table 4.6). Since changes in profit are susceptible to not only variation in cash crop yields but also market fluctuations, the variability of cover crop impacts on net returns likely represents a significant barrier to adoption and limits the viability of cover crops, especially in semi-arid regions.

Wheat-fallow systems in dryland regions already experience low net returns, leaving little additional profit to invest in conservation strategies like cover cropping. Though net returns of wheat-fallow control plots were positive on average in this study, it was negative in two of the five cycles across both trials and negative on average for T2. Net returns in dryland systems have a significant probability of being less than 0 due to variable precipitation and moisture limitations. A study in Akron, CO showed a 19% probability of negative returns in a wheat-fallow rotation, with this probability increasing for more intensified rotations (Nielsen et al., 2016b). Though the present research confirms the potential of cover crops to address soil degradation challenges, options must be explored to alleviate the financial burden that is associated with using cover crops as a fallow replacement.

The sale of 50% of cover crop biomass as forage appears to be a promising strategy to contribute to the economic viability of cover cropping. In four out of the five cropping cycles evaluated, including forage as an additional revenue source more than offset the costs and decreased wheat revenue associated with cover cropping, leading to overall increases in net returns as compared to the fallow treatment (Table 4.6). This is consistent with previous literature which has shown that grazing or harvesting cover crop biomass as forage can offset the cost of implementation in humid and water-limited systems alike (Holman et al., 2018; Plastina et al., 2018). Forage production also has the potential to provide economic resiliency for dryland systems in a changing climate, since yields are less sensitive to water stress during grain-filling developmental stages (Nielsen et al., 2010). A study in eastern CO showed that incorporation of forage into grain-based rotations yielded a greater likelihood for positive economic returns amidst variable precipitation and temperature patterns (Nielsen et al., 2016b). Forage crops have also been shown to have less impact on plant-available water at subsequent planting than grain crops and can be harvested early to lessen water use and increase soil moisture recharge before planting (Nielsen et al., 2017).

However, the production of sufficient biomass to support significant forage sales is necessary, and in water-limited systems, this is not always guaranteed. In Cycle 3 of T1, there was very little biomass production and forage revenue was not able to make up for reduced wheat revenue and direct costs (Table 4.6). Nielsen et al. (2017) also found that replacing fallow periods with forage production in dryland systems increased average net returns, but not in years with growing season precipitation that was far below average. Spring-planted plots also did not produce much biomass, and forage sales were not sufficient to offset reduced profit margins, which were especially large in these treatments due to additional costs for weed management in the fall and early spring.

It is important to note that our field trials did not include a treatment in which cover crop biomass was harvested, and ES associated with cover crops may not be as pronounced with partial biomass removal. To leave sufficient residue for supporting ES, our study considers only 50% removal of biomass in accordance with soil conservation recommendations (Natural Resources Conservation Service, 2021). Furthermore, soil health benefits have been observed in dryland cover crop plots with the harvesting or grazing of cover crop biomass (Kelly et al., 2021; Simon et al., 2022), and it is therefore reasonable to assume that erosion control and soil structure benefits would be comparable even with 50% biomass removal. We also note that wheat-fallow systems on the Colorado Plateau receive little to no fertilizer inputs, and partial harvest of cover crop biomass for forage would enhance nutrient export and may require additional nutrient inputs in the long-term that were not considered here.

Cost-share payments, such as the USDA Environmental Quality Incentives Program (EQIP), are another alternative to offsetting costs and increasing economic viability of cover crops and have been shown to increase likelihood of positive net returns in cover cropped systems (Plastina et al., 2018; Thompson et al., 2020). EQIP offers financial assistance to producers to adopt practices which provide off-farm environmental benefits, such as erosion control, water quality, C sequestration, among others (Natural Resources Conservation Service, 2022b). On the Colorado Plateau, an EQIP payment for the incorporation of cover crops is 118 USD ha^{-1} yr^{-1} (Natural Resources Conservation Service, 2022c), much less than the average decrease in net returns found here (176 USD ha^{-1} $cycle^{-1}$) that would be needed make farmers indifferent to the incorporation of cover crops. A combination of cost-share payments and forage sales would increase the probability of positive net profit due to cover crops, but harvesting cover crop biomass often disqualifies producers from participation in cost-share programs.

4.5 Conclusions

Trade-offs between ES associated with biomass production and ES linked to cash crop productivity have been observed in cover cropped systems in more humid climates, and our findings confirm that these relationships are even more pronounced in dryland systems. Erosion control and soil benefits from cover crops could help address soil degradation issues for dryland farmers on the Colorado Plateau, but the replacement of the fallow period decreases available moisture at planting and results in lowered and variable wheat yields. Spring-planted cover crops may balance this trade-off, though costs are high and soil health benefits may be too slow to justify, as trends were not significant after a six-year study period.

Wheat yield penalties and direct costs of cover cropping cause significant reductions in net returns, but the sale of fall-planted cover crop biomass as forage is a promising option that could more than compensate for these losses. Longer-term research is needed to further assess the viability of this option, as more pronounced soil health benefits may accrue over time, and economic impacts may change with fluctuations in hay and wheat prices. Shortening fallow periods in this region is imperative for sustained agricultural production, and our findings suggest that cover crop-based forage production may offer an economically and environmentally sustainable alternative.

Though cover cropping and other conservation practices are often widely promoted and incentivized, our research demonstrates that productivity trade-offs are likely, especially in water-limited systems, and these tradeoffs can lead to quite significant economic burdens at the farm-level. Given these trade-offs, comprehensive analysis of public benefits associated with regulating

ES and private costs associated with cash crop productivity should be conducted to determine whether practices are appropriate, and adequate cost-share payments or other incentives should be provided to ensure feasibility of adoption.

CHAPTER 4 TABLES AND FIGURES

Table 4.1. Cover crop (CC) treatments included in two adjacent dryland cover crop field trials (T1 and T2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Treatments are either a fall-planted CC mix, a spring-planted CC mix, or bare fallow rotated with winter wheat. Species for each CC mixture are listed followed by percent contribution (by seed weight) in parenthesis.

T1

CC Treatment	CC Planting Window	Species
Mix 1	Fall	Hairy Vetch[a] (14%), Yellow Sweet Clover[b] (3%), Winter Pea[c] (83%)
Mix 2	Fall	Hairy Vetch (8%), Yellow Sweet Clover (2%), Winter Pea (48%), Winter Rye[d] (43%)
Mix 3	Fall	Hairy Vetch (6%), Yellow Sweet Clover (1%), Winter Pea (34%), Winter Rye (53%), Winfred Turnip[e] (3%), Winter Canola[f] (3%)
Fallow	NA[1]	NA

T2[2]

CC Treatment	CC Planting Window	Species
Mix 1	Fall	Winter Pea (54%), Berseem Clover[g] (4%), Yellow Sweet Clover (1%), Winter Triticale[h] (35%), Forage Radish[i] (3%), Forage Turnip[j] (3%)
Mix 2	Fall	Winter Pea (55%), Lentil[k] (5%), Barley[l] (37%), Winter Canola (3%)
Mix 3	Fall	Winter Pea (50%), Hairy Vetch (10%), Winter triticale (17%), Oats[m] (17%), Forage radish (3%), Winter Canola (3%)
Mix 4	Fall	Winter Pea (59%), Hairy Vetch (19%), Winter triticale (7.5%), Oats (7.5%), Forage Radish (2.5%), Winter Canola (2.5%)
Mix 5	Fall	Winter Pea (32%), Hairy Vetch (8%), Winter triticale (28%), Oats (27%), Forage Radish (2.5%), Winter Canola (2.5%)
Mix 6	Spring	Hairy Vetch (15%), Spring Pea[n] (52%), Oats (20%), Winter Canola (1%), Forage radish (2%), Flax[o] (5%), [p]Safflower (5%)
Mix 7	Spring	Spring lentil[q] (45%), Berseem Clover[r] (15%), Oats (24%), German millet[s] (10%), Forage collards[t] (6%)
Mix 8	Spring	Spring pea (60%), Barley (40%)
Fallow	NA	NA

[1] NA, not applicable. [2] Cover crop mixtures were slightly modified in 2019 based on performance of species in early phases of the project and to reduce mixture cost. Cover crop mixtures planted in 2016 can be found in Eash et al. (2021). [a] *Vicia villosa* R. [b] *Melilotus officinalis* L. [c] *Pisum sativum* L. [d] *Secale cereale* L. [e] *Brassica napus* L., *cv.* Winfred [f] *Brassica napus* L. [g] *Trifolium alexandrinum* L. [h] *Triticosecale Wittm.* [i] *Raphanus sativus* L. [j] *Brassica rapa* L. [k] *Lens culinaris* L. [l] *Hordeum vulgare* L. [m]*Avena sativa* L. [n] *Pisum sativum* L. [n] *Avena sativa* L. [o] *Linum usitatissimum* L. [p] *Carthamus tinctorius* L. [q] *Lens culinaris* L. [r] *Trifolium alexandrinum* L. [s] *Panicum italicum* L. [t] *Brassica oleracea* L.

Table 4.2. Ecosystem services (ES) evaluated in two adjacent dryland cover crop field trials at the Southwestern Colorado Research Center near Yellow Jacket, Colorado.

ES	ES Proxy	ES category [1]
Crop production	Wheat yield	Provisioning
Yield Stability	Wheat yield stability	Provisioning
Forage production	Cover crop biomass	Provisioning
N supply	NO_3^- at wheat planting	Supporting
Soil water availability	Soil water storage at wheat planting	Supporting
Conservation of belowground biodiversity	Prokaryotic alpha diversity Prokaryotic richness	Supporting
Soil structure	Mean weight diameter of water-stable aggregates	Supporting
Erosion control	Soil loss	Regulating
C sequestration	Soil organic C	Regulating

[1] ES category defined by the Millennium Ecosystem Assessment (2005).

Table 4.3. Mean values of ecosystem service proxies for three cover cropping (CC) cycles at two adjacent dryland cover crop field trials (T1 and T2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Treatments consist of winter wheat rotated with either a fall-planted CC mix, a spring-planted CC mix, or bare fallow and managed under no-till (NT) or conventional tillage (CT). Values with different lowercase letters (by column) indicate differences to an alpha level of 0.05, as determined by Tukey-adjusted multiple comparisons.

T1

		Cycle 1				Cycle 2				Cycle 3			
Tillage	CC	Cover Crop Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield	Cover Crop Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield	Cover Crop Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield
		kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}	kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}	kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}
NT	Fall	5020	9.93 a	157 a	2.95 a	1510	12.4 a	123 a	1.52 a	1210	3.75 a	134 a	0.22 a
	Fallow	NE[1]	17.1 b	224 b	4.03 b	NE	19.4 b	131 a	1.35 b	NE	6.04 b	176 b	1.14 b
		ANOVA[2]											
		NA	**<0.001**	**<0.001**	**<0.001**	NA	**0.001**	0.24	**0.009**	NA	**0.038**	**<0.001**	**<0.001**

T2

		Cycle 1				Cycle 2				Cycle 3[4]		
Tillage	CC	Cover Crop Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield	Cover Crop Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield	Cover Crop Biomass	Soil Nitrate	Soil Water Storage
		kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}	kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}	kg ha^{-1}	mg kg^{-1}	mm
NT	Fall	3880 a	4.36 ab	148 ac	0.44 ab	4700 a	1.09 a	111 a	0.54 ac	1500 a	3.07 a	120 a
	Spring	637 b	5.43 b	179 bd	1.19 cd	2100 b	1.58 a	128 ab	0.85 bde	950 b	5.77 a	115 a
	Fallow	NE	9.61 c	209 d	1.60 d	NE	3.43 b	156 c	1.26 fg	NE	9.36 a	169 b
CT	Fall	3820 a	3.14 a	143 ab	0.14 a	4840 a	0.77 a	118 a	0.52 ab	1340 a	6.52 a	121 a
	Spring	845 b	5.34 b	185 cd	0.81 bc	2050 b	1.58 a	132 abc	0.93 cdf	104 c	7.70 a	136 a b
	Fallow	NE	9.15 c	211 d	0.95 bcd	NE	1.25 a	153 bc	1.17 eg	NE	5.91 a	142 a b
		ANOVA[3]										
	CC	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.13	**0.002**
	Tillage	0.79	0.11	0.89	0.059	0.88	0.073	0.7	0.94	**0.02**	0.65	0.82
	CC x Tillage	0.57	0.1	0.72	0.19	0.72	**0.0038**	0.58	0.13	**<0.001**	0.09	0.063

[1] NE, not evaluated. [2] Differences analyzed using a multifactor analysis of variance (ANOVA) with CC included as a fixed effect and block included as a random effect. *p*-values presented and bolded when significant at an alpha level of 0.05. [3] Differences analyzed using a multifactor ANOVA with CC, tillage and interaction included as fixed effects and block and tillage subplots included as random effects. *p*-values presented and bolded when significant at an alpha level of 0.05. [4] Wheat yield not collected in cycle 3 of T2, as project ended in Spring 2022.

Table 4.4. Cumulative values of provisioning (PROV), supporting (SUPP), and regulating (REG) ecosystem service indices and proxies following three cover cropping (CC) cycles at two adjacent dryland cover crop field trials (T1 and T2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Treatments consist of winter wheat rotated with either a fall-planted CC mix, a spring-planted CC mix, or bare fallow and managed under no-till (NT) or conventional tillage (CT). Values with different lowercase letters (by column) indicate differences to an alpha level of 0.05, as determined by Tukey-adjusted multiple comparisons.

T1

Tillage	CC	PROV	Wheat Yield	Yield Stability	CC Biomass	SUPP	Aggregation (MWD [1])	Alpha Diversity	Soil Water Storage	Soil Nitrate	REG	Soil loss	SOC
			Mg ha^{-1}		kg ha^{-1}		µm	Shannon Index	mm	mg kg^{-1}		kg ha^{-1} yr^{-1}	%
NT	Fall	0.435	1.56	0.13	2580	0.470	233	7.4	135	6.56	0.788	220	0.8
	Fallow	0.619	2.18	0.26	NE [2]	0.568	185	7.33	170	10.16	0.276	568	0.8
							ANOVA [3]						
	CC	**0.0074**	**<0.001**	**0.002**	**<0.001**	**0.040**	**0.05**	0.30	<0.001	<0.001	**<0.001**	NA [4]	0.5

T2

Tillage	CC	PROV	Wheat Yield [5]	Yield Stability	CC Biomass	SUPP	Aggregation (MWD)	Alpha Diversity	Soil Water Storage	Soil Nitrate	REG	Soil loss	SOC
			Mg ha^{-1}		kg ha^{-1}		µm	Shannon Index	mm	mg kg^{-1}		kg ha^{-1} yr^{-1}	%
NT	Fall	0.630 a	0.49 ab	0.69 a	3360 a	0.415 a	276 a	7.49 a	115 a	2.05 ab	0.783 a	188	0.67
	Spring	0.611 a	1.02 c	0.77 a	1230 b	0.428 a	236 ab	7.46 a	121 a	3.99 b	0.707 a	346	0.69
	Fallow	0.626 a	1.43 d	0.84 a	NE	0.649 b	203 b	7.56 a	162 c	4.56 b	0.730 a	568	0.70
CT	Fall	0.472 b	0.33 a	0.12 b	3330 a	0.460 ab	287 a	7.50 a	120 a	1.52 a	0.701 a	568	0.68
	Spring	0.575 a	0.87 bc	0.82 a	999 b	0.521 ab	233 ab	7.59 a	134 ab	3.92 b	0.388 b	2619	0.71
	Fallow	0.549 ab	1.06 cd	0.83 a	NE	0.596 ab	240 ab	7.52 a	147 bc	3.65 ab	0.277 b	7289	0.62
							ANOVA [6]						
	CC	**0.038**	**<0.001**	**<0.001**	**<0.001**	**0.0014**	**<0.001**	0.78	**<0.001**	**<0.001**	**<0.001**	NA [3]	0.56
	Tillage	**0.021**	0.15	0.081	0.68	0.49	0.32	0.69	0.78	0.38	**0.012**	NA	0.67
	CC x Tillage	**0.0026**	0.18	**<0.001**	0.64	0.31	0.54	0.43	**0.027**	0.82	**<0.001**	NA	0.38

[1] MWD, mean weight diameter. [2] NE, not evaluated. [3] Differences analyzed using a multifactor analysis of variance (ANOVA) with CC included as a fixed effect and block included as a random effect. *p*-values presented and bolded when significant at an alpha level of 0.05. [4] NA, not applicable. Statistical analysis not performed on modelled soil loss estimates. [5] T2 only includes wheat yield data from two cropping cycles. [6] Differences analyzed using a multifactor ANOVA with CC, tillage and interaction included as fixed effects and block and tillage subplots included as random effects. *p*-values presented and bolded when significant at an alpha level of 0.05.

Table 4.5. Costs, revenue, and net returns following three cover crop (CC) – winter wheat cycles at two adjacent dryland cover crop field trials (T1 and T2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Treatments consist of winter wheat rotated with either a fall-planted CC mix, a spring-planted CC mix, or bare fallow and managed under no-till (NT) or conventional tillage (CT). Net returns were evaluated with and without the sale of 50% of CC biomass as forage. All values are displayed in USD ha^{-1} cycle^{-1}.

			Net Returns without Forage				Net Returns with Forage				Average Wheat and CC Costs	Average Wheat Revenue	Average Additional Forage Costs	Average Forage Revenue
Trial	Tillage	CC	Cycle 1	Cycle 2	Cycle 3	Average	Cycle 1	Cycle 2	Cycle 3	Average				
T1	NT	Fall	-10.16	-66.76	-193.33	-90.08	377.03	6.61	-134.83	82.94	272.23	182.14	82.03	255.05
		Fallow	209.99	-8.59	80.39	93.93	209.99	-8.59	80.39	93.93	187.29	281.22	NA [1]	NA
T2	NT	Fall	-198.09	-157.44	NA	-177.77	78.39	226.52	NA	152.45	236.20	58.43	87.26	417.48
		Spring	-143.05	-156.19	NA	-149.62	-181.97	-25.69	NA	-103.83	269.43	119.81	87.26	133.05
		Fallow	-18.85	1.31	NA	-8.77	-18.85	1.31	NA	-8.77	171.68	162.91	NA	NA
	CT	Fall	-222.96	-128.25	NA	-175.61	48.07	269.11	NA	158.59	217.99	42.38	87.26	421.45
		Spring	-134.16	-93.34	NA	-113.75	-152.85	32.38	NA	-60.24	217.68	103.94	87.26	140.77
		Fallow	-56.75	22.27	NA	-17.24	-56.75	22.27	NA	-17.24	143.23	125.99	NA	NA

[1] NA, not applicable.

Table 4.6. Change in net returns due to the replacement of fallow with fall- and spring-planted cover crop (CC) treatments, managed under either no-till (NT) or conventional tillage (CT). Data are from three cover crop (CC) – winter wheat cycles at two adjacent dryland cover crop field trials (T1 and T2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Net returns were evaluated both excluding and including the sale of 50% of CC biomass as forage. All values are displayed in USD ha^{-1} cycle^{-1}.

Forage Revenue	Trial	Tillage	CC	Change in net returns Cycle 1	Cycle 2	Cycle 3	Average
Excluded	T1	NT	Fall	-220.15	-58.17	-273.71	-184.01
	T2	NT	Fall	-179.24	-158.75	NA	-169.00
			Spring	-124.20	-157.51	NA	-140.85
		CT	Fall	-166.21	-150.52	NA	-158.37
			Spring	-77.41	-115.60	NA	-96.51
Included	T1	NT	Fall	167.04	15.20	-215.21	-10.99
	T2	NT	Fall	97.24	225.21	NA	161.22
			Spring	-163.12	-27.00	NA	-95.06
		CT	Fall	104.81	246.84	NA	175.83
			Spring	-96.10	10.11	NA	-43.00

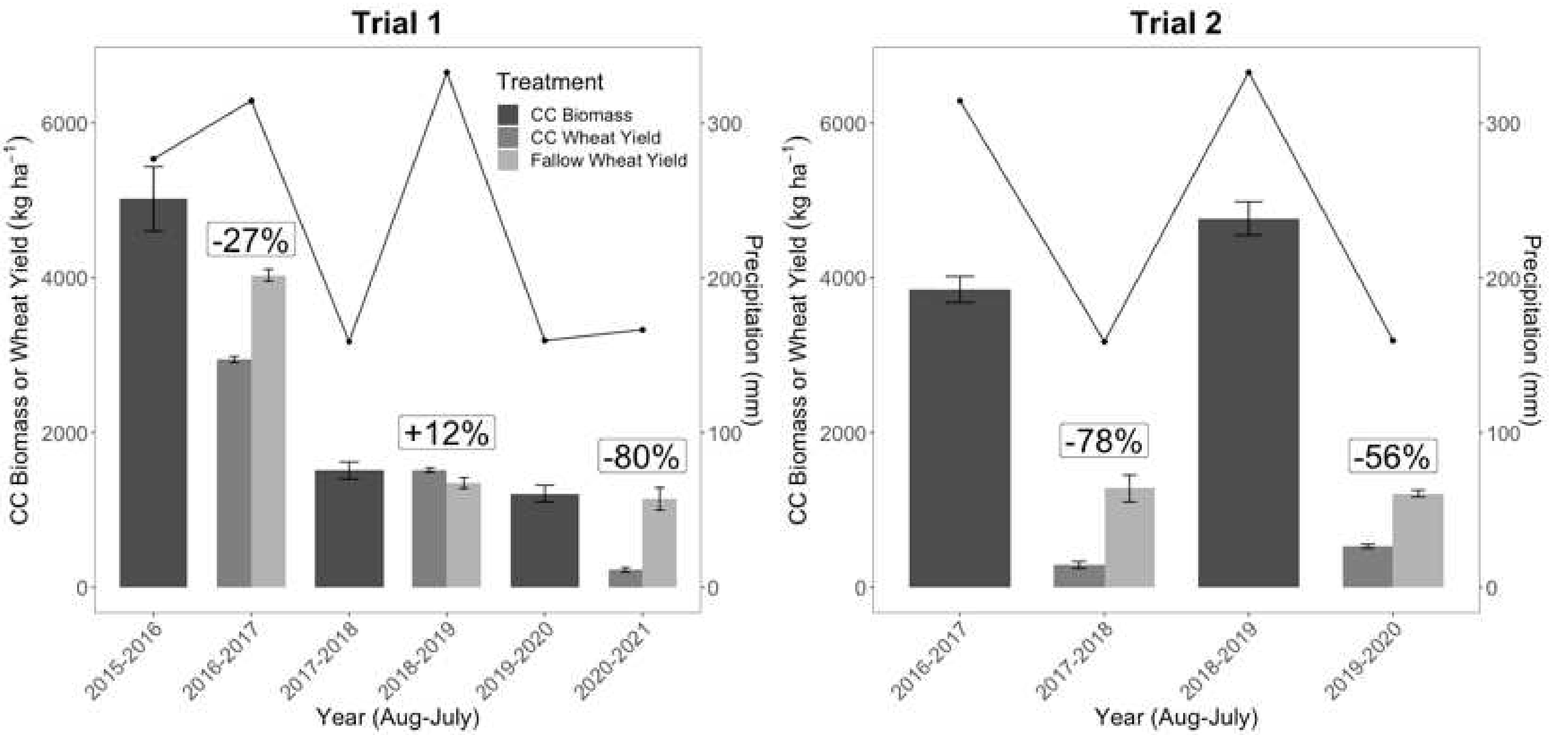

Figure 4.1. Winter wheat yield penalties shown alongside fall-planted cover crop (CC) biomass production and yearly precipitation (August through July; designated by line and secondary y-axis) from two adjacent dryland cover crop field trials (Trial 1 and Trial 2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. The darkest bars indicate fall-planted CC biomass in alternate years, medium-gray bars indicate wheat yields following CC, and light-gray bars indicate wheat yields following a fallow period. Yield penalties are in text boxes displayed above wheat yields and were calculated as the percent reduction or increase of wheat yields following CC as compared to fallow control plots.

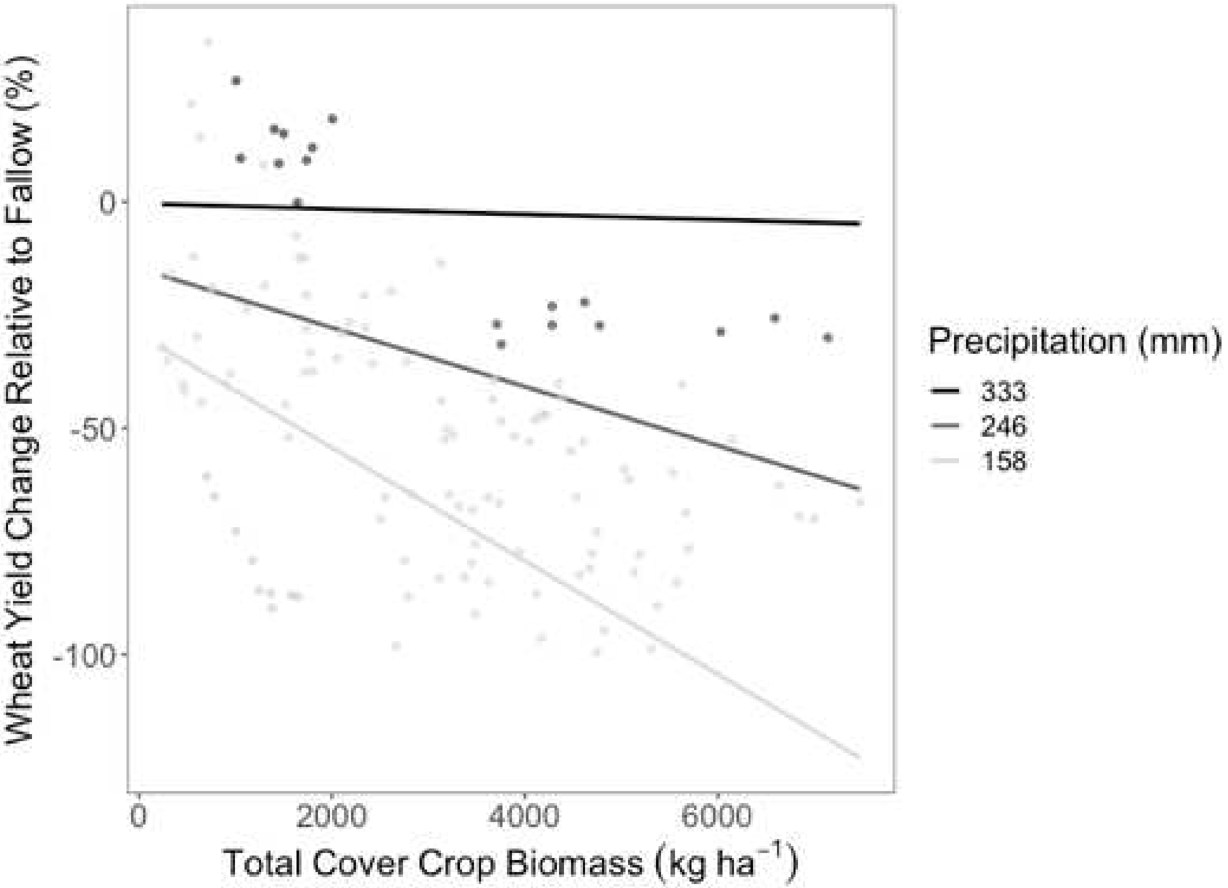

Figure 4.2. Changes in winter wheat yield following cover crop (CC) relative to wheat yields following a 14-month fallow period depend on an interactive effect between CC biomass production and growing season precipitation (*p* = 0.023). Growing season precipitation was received between August and July during the year of wheat growth. Regression lines are shown for a range of precipitation levels observed in the study period. Data are from three CC cycles at two adjacent dryland cover crop field trials (T1 and T2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado.

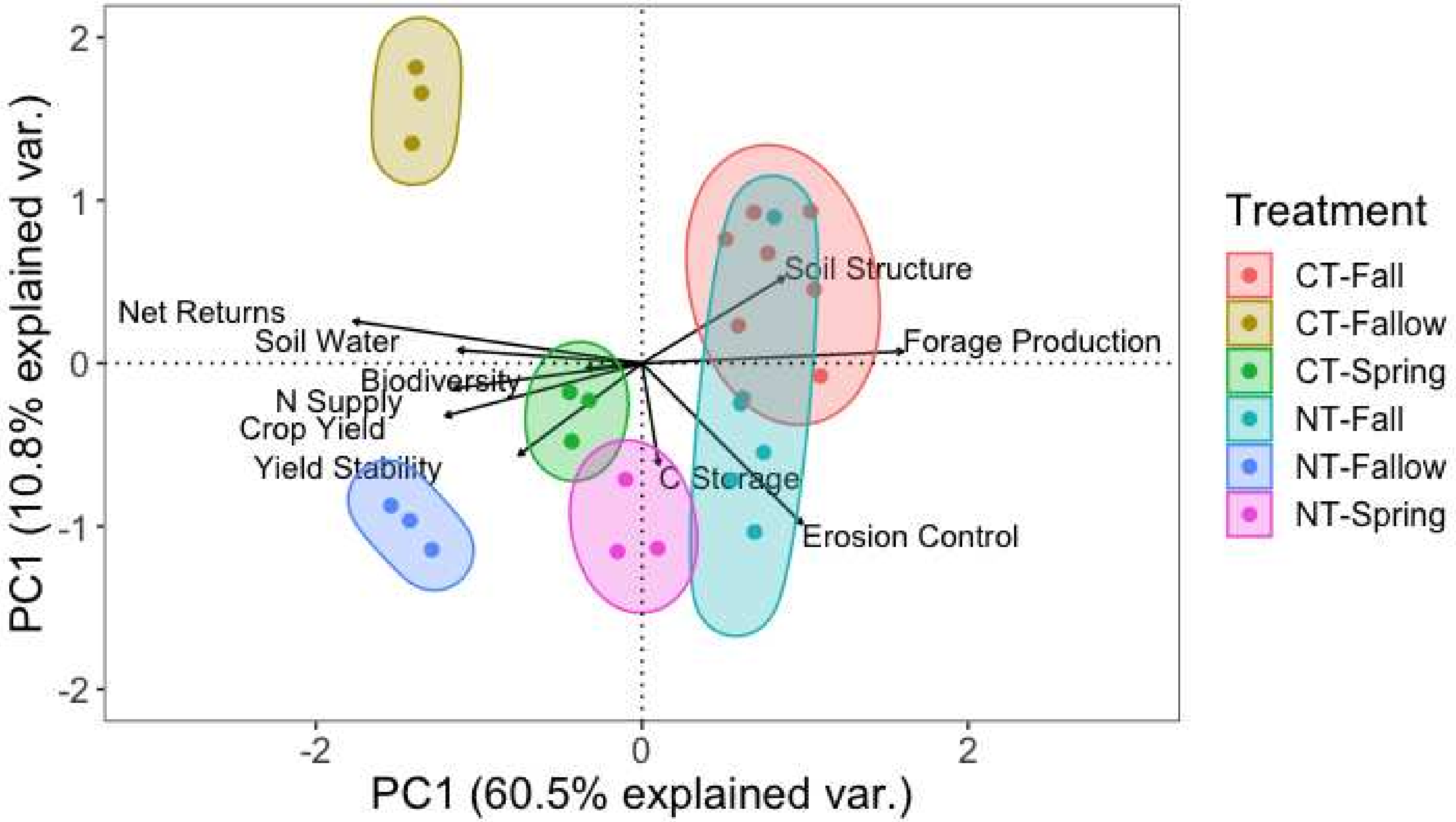

Figure 4.3. Principal component analysis (PCA) ordination of ecosystem services from a dryland cover crop (CC) field trial (T2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Treatments consist of winter wheat rotated with either a fall-planted CC mix, a spring-planted CC mix, or bare fallow and managed under no-till (NT) or conventional tillage (CT). Ecosystem service values are averaged over two CC cycles.

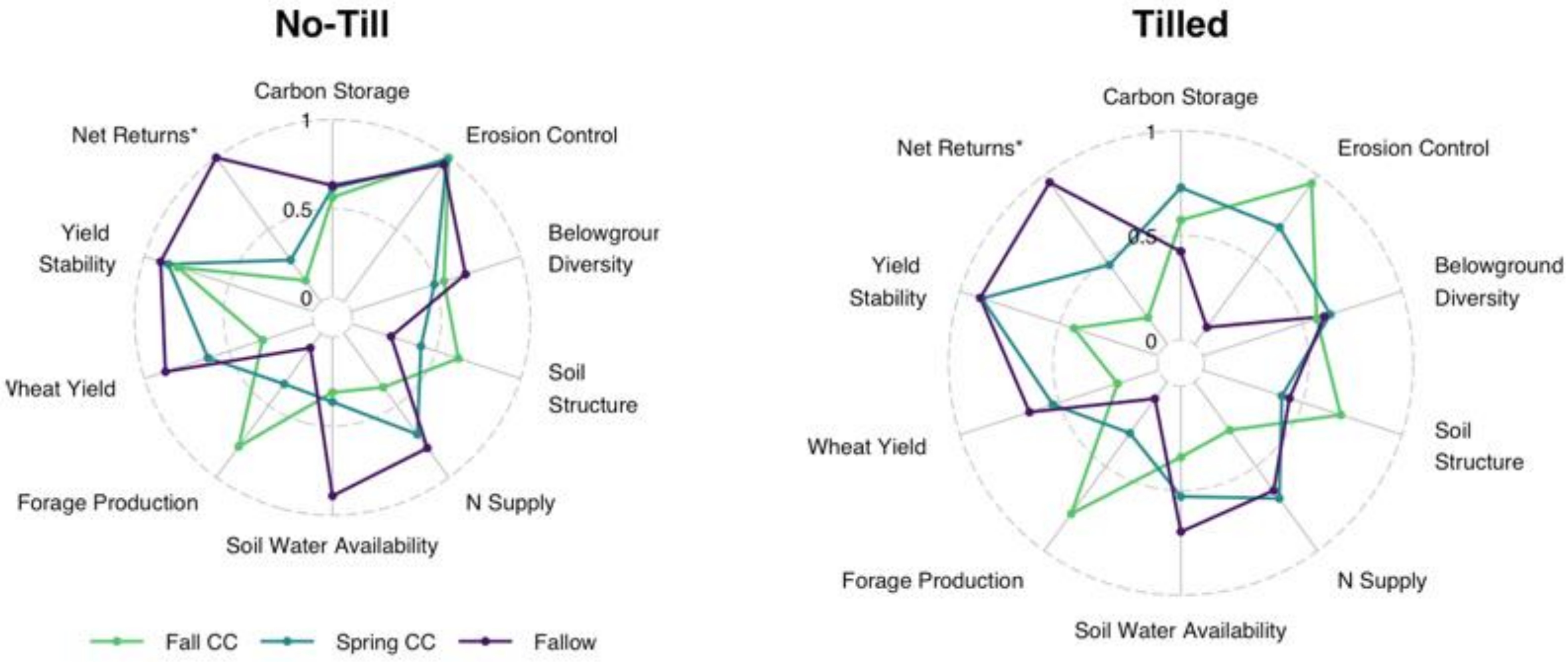

Figure 4.4. Radar plots displaying multifunctionality of fall- and spring-planted cover crop (CC) treatments, managed under either no-till or conventional tillage. Data are from two cover crop (CC) – winter wheat cycles at a dryland cover crop field trial (T2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. *Note that net returns displayed here do not include the hypothetical sale of cover crops as forage for additional revenue.

REFERENCES

Adusumilli, N., Wang, H., Dodla, S., & Deliberto, M. (2020). Estimating risk premiums for adopting no-till and cover crops management practices in soybean production system using stochastic efficiency approach. *Agricultural Systems, 178*. https://doi.org/10.1016/j.agsy.2019.102744.

Aiken, R. M., O'Brien, D. M., Olson, B. L., & Murray, L. (2013). Replacing fallow with continuous cropping reduces crop water productivity of semiarid wheat. *Agronomy Journal, 105*, 199–207. https://doi.org/10.2134/agronj2012.0165.

Agricultural Marketing Service. (2017). *Colorado Hay Report.* U.S. Department of Agriculture. https://mymarketnews.ams.usda.gov/filerepo/sites/default/files/2905/2017-11-09/397937/GL_GR31020171109.TXT

Agricultural Marketing Service. (2019). *Colorado Hay Report.* U.S. Department of Agriculture. https://mymarketnews.ams.usda.gov/filerepo/sites/default/files/2905/2019-08-15/87720/GL_GR31020190815.TXT

Agricultural Marketing Service. (2021). *Colorado Direct Hay Report.* U.S. Department of Agriculture. https://mymarketnews.ams.usda.gov/filerepo/sites/default/files/2905/2021-06-28/465172/ams_2905_00068.pdf

Agricultural Research Service. (2020). *Wind Erosion Prediction System (WEPS): Technical Documentation*. In Tatarko, J. (Ed.) Agriculture Handbook 727. U.S. Department of Agriculture. https://www.nrcs.usda.gov/wps/PA_NRCSConsumption/download?cid=nrcseprd1653618&ext=pdf

Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Angel, R., Pasternak, Z., Soares, M. I. M., Conrad, R., & Gillor, O. (2013). Active and total prokaryotic communities in dryland soils. *FEMS Microbiology Ecology, 86*(1), 130-138. https://doi.org/10.1111/1574-6941.12155.

Barthes, B., & Roose, E. (2002). Aggregate stability as an indicator of soil susceptibility to runoff and erosion; validation at several levels. *Catena, 47*(2), 133-149. https://doi.org/10.1016/S0341-8162(01)00180-1.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01.

Bergtold, J. S., Ramsey, S., Maddy, L., & Williams, J. R. (2019). A review of economic considerations for cover crops as a conservation practice. *Renewable Agriculture and Food Systems*, *34*(1), 62-76. https://doi.org/10.1017/S1742170517000278.

Blanco-Canqui, H., Holman, J. D., Schlegel, A. J., Tatarko, J., & Shaver, T. M. (2013). Replacing fallow with cover crops in a semiarid soil: Effects on soil properties. *Soil Science Society of America Journal*, *77*(3), 1026-1034. https://doi.org/10.2136/sssaj2013.01.0006.

Blevins, R. L., Smith, M. S., Thomas, G. W., & Frye, W. W. (1983). Influence of conservation tillage on soil properties. *Journal of soil and water conservation*, *38*(3), 301-305.

Bronick, C.J., & Lal, R. (2005). Soil structure and management: a review. *Geoderma*, *124*, 3-22. https://doi.org/10.1016/j.geoderma.2004.03.005.

Campbell, C. A., Janzen, H. H., Paustian, K., Gregorich, E. G., Sherrod, L., Liang, B. C., & Zentner, R. P. (2005). Carbon storage in soils of the North American Great Plains: Effect of cropping frequency. *Agronomy Journal*, *97*(2), 349-363. https://doi.org/10.2134/agronj2005.0349.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., ... & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME journal*, *6*(8), 1621-1624. https://doi.org/10.1038/ismej.2012.8.

Colorado State University Extension. (2016). *Crop Enterprise Budget - Western Winter Wheat (Dryland)*. https://extension.colostate.edu/docs/abm/westerndrylandwinterwheat16.pdf

Colorado State University Extension. (2018). C*rop Enterprise Budget - Western Winter Wheat (Dryland).* https://extension.colostate.edu/docs/abm/westerndrylandwinterwheat18.pdf =

Colorado State University Extension. (2021). *Crop Enterprise Budget - Western Winter Wheat (Dryland).* https://abm.extension.colostate.edu/wp-content/uploads/sites/61/2022/04/2021-W-DrylandWinterWheat.xlsx

Dabney, S. M., Delgado, J. A., & Reeves, D. W. (2001). Using winter cover crops to improve soil and water quality. *Communications in Soil Science and Plant Analysis*, *32*(7-8), 1221-1250. https://doi.org/10.1081/CSS-100104110.

Daryanto, S., Fu, B., Wang, L., Jacinthe, P. A., & Zhao, W. (2018). Quantitative synthesis on the ecosystem services of cover crops. *Earth-Science Reviews*, *185*, 357-373. https://doi.org/10.1016/j.earscirev.2018.06.013.

Eash, L., Berrada, A. F., Russell, K., & Fonte, S. J. (2021). Cover crop impacts on water dynamics and yields in dryland wheat systems on the Colorado Plateau. *Agronomy Journal, 11*(6), 1102. https://doi.org/10.3390/agronomy11061102.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics, 26*(19), 2460-2461. https://doi.org/10.1093/bioinformatics/btq461.

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature methods*, *10*(10), 996-998. https://doi.org/10.1038/nmeth.2604.

Edgar, R. C. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *BioRxiv*, 081257. https://doi.org/10.1101/081257.

Elliott, E. T. (1986). Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. *Soil science society of America journal*, *50*(3), 627-633. https://doi.org/10.2136/ sssaj1986.03615995005000030017x.

Fan, K., Delgado-Baquerizo, M., Guo, X., Wang, D., Zhu, Y. G., & Chu, H. (2021). Biodiversity of key-stone phylotypes determines crop production in a 4-decade fertilization experiment. *The ISME journal*, *15*(2), 550-561. https://doi.org/10.1038/s41396-020-00796-8.

Finney, D. M., White, C. M., & Kaye, J. P. (2016). Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. *Agronomy Journal*, *108*(1), 39-52. https://doi.org/10.2134/agronj15.0182.

Fryrear, D. W. (1981). Dust storms in the southern Great Plains. *Transactions of the ASAE*, *24*(4), 991-0994. https://doi.org/10.13031/2013.34379.

García-González, I., Quemada, M., Gabriel, J. L., & Hontoria, C. (2016). Arbuscular mycorrhizal fungal activity responses to winter cover crops in a sunflower and maize cropping system. *Applied Soil Ecology*, *102*, 10-18. https://doi.org/10.1016/j.apsoil.2016.02.006.

Ghimire, R., Ghimire, B., Mesbah, A. O., Idowu, O. J., O'Neill, M. K., Angadi, S. V., & Shukla, M. K. (2018). Current status, opportunities, and challenges of cover cropping for sustainable dryland farming in the Southern Great Plains. *Journal of Crop Improvement*, *32*(4), 579-598. https://doi.org/10.1080/15427528.2018.1471432.

Hansen, N. C., Allen, B. L., Baumhardt, R. L., & Lyon, D. J. (2012). Research achievements and adoption of no-till, dryland cropping in the semi-arid US Great Plains. *Field Crops Research*, *132*, 196-203. https://doi.org/10.1016/j.fcr.2012.02.021.

Holman, J. D., Arnet, K., Dille, J., Maxwell, S., Obour, A., Roberts, T., ... & Schlegel, A. (2018). Can cover or forage crops replace fallow in the semiarid Central Great Plains? *Crop Science*, *58*(2), 932-944. https://doi.org/10.2135/cropsci2017.05.0324.

Hunter, M. C., Schipanski, M. E., Burgess, M. H., LaChance, J. C., Bradley, B. A., Barbercheck, M. E., ... & Mortensen, D. A. (2019). Cover crop mixture effects on maize, soybean, and wheat yield in rotation. *Agricultural & Environmental Letters*, *4*(1), 180051. https://doi.org/10.2134/ael2018.10.0051.

Jeranyama, P., & Garcia, A. D. (2004). Understanding relative feed value (RFV) and relative forage quality (RFQ). *SDSU Extension Extra Archives*. 352. https://openprairie.sdstate.edu/extension_extra/352

Kearney, S. P., Fonte, S. J., García, E., Siles, P., Chan, K. M. A., & Smukler, S. M. (2019). Evaluating ecosystem service trade-offs and synergies from slash-and-mulch agroforestry systems in El Salvador. *Ecological indicators*, *105*, 264-278. https://doi.org/10.1016/j.ecolind.2017.08.032.

Keeney, D. R., & Nelson, D. W. (1982). *N-Inorganic Forms.* In Page, A.L. (Ed.), Methods of Soil Analysis, Agronomy Monograph 9, Part 2. ASA, SSSA. Madison, WI, pp. 643-698.

Kelly, C., Schipanski, M. E., Tucker, A., Trujillo, W., Holman, J. D., Obour, A. K., Johnson, S. K., Brummer, J. E., Haag, L., & Fonte, S. J. (2021). Dryland cover crop soil health benefits are maintained with grazing in the US High and Central Plains. *Agriculture, Ecosystems & Environment*, *313*, 107358. https://doi.org/10.1016/j.agee.2021.107358.

Kessavalou, A., & Walters, D. T. (1997). Winter rye as a cover crop following soybean under conservation tillage. *Agronomy Journal*, *89*(1), 68-74. https://doi.org/10.2134/agronj1997.00021962008900010011x.

Kim, N., Zabaloy, M. C., Guan, K., & Villamil, M. B. (2020). Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biology and Biochemistry*, *142*, 107701. https://doi.org/10.1016/j.soilbio.2019.107701.

Larson, J. A., Jaenicke, E. C., Roberts, R. K., & Tyler, D. D. (2001). Risk effects of alternative winter cover crop, tillage, and nitrogen fertilization systems in cotton production. *Journal of Agricultural and Applied Economics*, *33*(3), 445-457. https://doi.org/10.1017/S1074070800030182

Lenth, R., Buerkner, P., Herve, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2022). Emmeans: Estimated marginal means, aka least-squares means. R Package Version, 1.7.4-1.

Liebig, M. A., Tanaka, D. L., & Wienhold, B. J. (2004). Tillage and cropping effects on soil quality indicators in the northern Great Plains. *Soil and Tillage Research*, *78*(2), 131-141. https://doi.org/10.1016/j.still.2004.02.002.

Lyon, D. J., Baltensperger, D. D., Blumenthal, J. M., Burgener, P. A., & Harveson, R. M. (2004). Eliminating summer fallow reduces winter wheat yields, but not necessarily system profitability. *Crop Science*, *44*(3), 855-860. https://doi.org/10.2135/cropsci2004.0855.

Maestre, F.T., Delgado-Baquerizo, M., Jeffries, T.C., Eldridge, D.J., Ochoa, V., Gozalo, B., Quero, J.L., Garcia-Gomez, M., Gallardo, A., Ulrich, W., Bowker, M.A., Arredondo, T., Barraza-Zepeda, C., Bran, D., Florentino, A., Gaitan, J., Gutierrez, J.R., Huber-Sannwald, E., Jankju, M., Mau, R.L., Miriti, M., Naseri, K., Ospina, A., Stavi, I., Wang, D., Woods, N.N., Yuan, X., Zaady, E., & Singh, B. K. (2015). Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences*, *112*(51), 15684-15689. https://doi.org/10.1073/pnas.1516684112.

Maron, P.A., Sarr, A., Kaisermann, A., Lévêque, J., Mathieu, O., Guigue, J., Karimi, B., Bernard, L., Dequiedt, S., Terrat, S., Chabbi, A., & Ranjard, L. (2018). High microbial diversity promotes soil ecosystem functioning. *Applied and Environmental Microbiology*, *84*(9), e02738-17. https://doi.org/10.1128/AEM.02738-17.

Marsalis, M.A., Hagevoort, G.R., & Lauriault, L.M. (2009). *Hay Quality, Sampling & Testing*. New Mexico State University Cooperative Extension Service. https://pubs.nmsu.edu/_circulars/CR641

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal*, *17*(1), 10-12. https://doi.org/10.14806/ej.17.1.200.

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*(3), e02278. https://doi.org/10.1002/eap.2278.

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS one*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217.

Millennium Ecosystem Assessment. (2005). Ecosystems and Human Well-Being: Synthesis. Island Press, Washington, DC.

Miller, P. R., Lighthiser, E. J., Jones, C. A., Holmes, J. A., Rick, T. L., & Wraith, J. M. (2011). Pea green manure management affects organic winter wheat yield and quality in semiarid Montana. *Canadian Journal of Plant Science*, *91*(3), 497-508. https://doi.org/10.4141/cjps10109.

Natural Resources Conservation Service. (2001). *Revised Universal Soil Loss Equation Version 2 Handbook*. U.S. Department of Agriculture. https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs144p2_025079.pdf

Natural Resources Conservation Service. (2021). *Conservation Practice Standard: Cover Crop.* U.S. Department of Agriculture. https://www.blogs.nrcs.usda.gov/Internet/FSE_DOCUMENTS/16/nrcseprd1740246.pdf

Natural Resources Conservation Service. (2022a). *USDA offers expanded conservation program opportunities to support Climate Smart Agriculture in 2022.* U.S. Department of Agriculture. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/national/newsroom/releases/?cid=NRCSEPRD1867846

Natural Resources Conservation Service. (2022b). *Environmental Quality Incentives Program*. U.S. Department of Agriculture. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/co/home/?cid=stelprdb1044009

Natural Resources Conservation Service. (2022c). *Colorado Payment Schedules: Environmental Quality Incentives Program. Fiscal Year 2022.* U.S. Department of Agriculture. https://www.nrcs.usda.gov/wps/PA_NRCSConsumption/download?cid=NRCSEPRD1853861&ext=pdf

Nielsen, D. C., Halvorson, A. D., & Vigil, M. F. (2010). Critical precipitation period for dryland maize production. *Field Crops Research*, *118*(3), 259-263. https://doi.org/10.1016/j.fcr.2010.06.004.

Nielsen, D. C., Lyon, D. J., Higgins, R. K., Hergert, G. W., Holman, J. D., & Vigil, M. F. (2016). Cover crop effect on subsequent wheat yield in the central Great Plains. *Agronomy Journal*, *108*(1), 243-256. https://doi.org/10.2134/agronj2015.0372.

Nielsen, D. C., Lyon, D. J., & Miceli-Garcia, J. J. (2017). Replacing fallow with forage triticale in a dryland wheat-corn-fallow rotation may increase profitability. *Field Crops Research*, *203*, 227-237. https://doi.org/10.1016/j.fcr.2016.12.005.

Nielsen, D. C., & Vigil, M. F. (2005). Legume green fallow effect on soil water content at wheat planting and wheat yield. *Agronomy Journal*, *97*(3), 684-689. https://doi.org/10.2134/agronj2004.0071.

Nielsen, D. C., & Vigil, M. F. (2018). Wheat yield and yield stability of eight dryland crop rotations. *Agronomy Journal*, *110*(2), 594-601. https://doi.org/10.2134/agronj2017.07.0407.

Nielsen, D. C., Vigil, M. F., & Hansen, N. C. (2016). Evaluating potential dryland cropping systems adapted to climate change in the central Great Plains. *Agronomy Journal*, *108*(6), 2391-2405. https://doi.org/10.2134/agronj2016.07.0406.

Ortega, R. A., Peterson, G. A., & Westfall, D. G. (2002). Residue accumulation and changes in soil organic matter as affected by cropping intensity in no-till dryland agroecosystems. *Agronomy Journal*, *94*(4), 944-954. https://doi.org/10.2134/agronj2002.9440.

Peterson, G. A., Schlegel, A. J., Tanaka, D. L., & Jones, O. R. (1996). Precipitation use efficiency as affected by cropping and tillage systems. *Journal of Production Agriculture*, *9*(2), 180-186. https://doi.org/10.2134/jpa1996.0180.

Peterson, G. A., & Westfall, D. G. (2004). Managing precipitation use in sustainable dryland agroecosystems. *Annals of Applied Biology*, *144*(2), 127-138. https://doi.org/10.1111/j.1744-7348.2004.tb00326.x.

Peterson, G. A., Westfall, D. G., & Cole, C. V. (1993). Agroecosystem approach to soil and crop management research. *Soil Science Society of America Journal*, *57*(5), 1354-1360. https://doi.org/10.2136/sssaj1993.03615995005700050032x.

Peterson, G. A., Westfall, D. G., Schipanski, M. E., & Fonte, S. J. (2020). Soil and crop management systems that ameliorate damage caused by decades of dryland agroecosystem mismanagement. *Agronomy Journal*, *112*(5), 3227-3238. https://doi.org/10.1002/agj2.20257.

Pilgrim, E.S., Macleod, C.J.A., Blackwell, M.S.A., Bol, R., Hogan, D.V., Chadwick, D.R., Cardenas, L., Misselbrook, T.H., Haygarth, P.M., Brazier, R.E., Hobbs, P., Hodgson, C., Jarvis, S., Dungait, J., Murray, P.J., & Firbank, L.G. (2010). Interactions among agricultural production and other ecosystem services delivered from European temperate grassland systems. *Advances in agronomy*, *109*, 117-154. https://doi.org/10.1016/B978-0-12-385040-9.00004-9.

Plastina, A. (2016). Estimated costs of crop production in Iowa. Iowa State University Extension and Outreach. https://www.extension.iastate.edu/agdm/crops/pdf/a1-20_2016.pdf

Plastina, A. (2018). *Estimated costs of crop production in Iowa*. Iowa State University Extension and Outreach. https://www.extension.iastate.edu/agdm/crops/pdf/a1-20_2018.pdf

Plastina, A. (2020). *Estimated costs of crop production in Iowa*. Iowa State University Extension and Outreach. https://www.extension.iastate.edu/agdm/crops/pdf/a1-20-2020.pdf

Plastina, A., Liu, F., Sawadgo, W., Miguez, F. E., Carlson, S., & Marcillo, G. (2018). Annual net returns to cover crops in Iowa. *Journal of Applied Farm Economics*, *2*(2). https://doi.org/10.7771/2331-9151.1030.

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

Ponderosa Partnership. (2018). Ponderosa Hay. https://www.ponderosapartnership.com/forage-crops

PRISM Climate Group. (2014). Oregon State University. https://prism.oregonstate.edu (accessed 21 April 2022).

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., & Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic acids research*, *35*(21), 7188-7196. https://doi.org/10.1093/nar/gkm864.

R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Reese, C. L., Clay, D. E., Clay, S. A., Bich, A. D., Kennedy, A. C., Hansen, S. A., & Moriles, J. (2014). Winter cover crops impact on corn production in semiarid regions. *Agronomy Journal*, *106*(4), 1479-1488. https://doi.org/10.2134/agronj13.0540.

Saseendran, S. A., Nielsen, D. C., Ahuja, L. R., Ma, L., & Lyon, D. J. (2013). Simulated yield and profitability of five potential crops for intensifying the dryland wheat-fallow production system. *Agricultural Water Management*, *116*, 175-192. https://doi.org/10.1016/j.agwat.2012.07.009.

Schipanski, M.E., Barbercheck, M., Douglas, M.R., Finney, D.M., Haider, K., Kaye, J.P., Kemanian, A.R., Mortensen, D.A., Ryan, M.R., Tooker, J., & White, C. (2014). A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agricultural Systems*, *125*, 12-22. https://doi.org/10.1016/j.agsy.2013.11.004.

Schwinning, S., Belnap, J., Bowling, D. R., & Ehleringer, J. R. (2008). Sensitivity of the Colorado Plateau to change: climate, ecosystems, and society. *Ecology and Society*, *13*(2).

Shaver, T. M., Peterson, G. A., Ahuja, L. R., Westfall, D. G., Sherrod, L. A., & Dunn, G. (2002). Surface soil physical properties after twelve years of dryland no-till management. *Soil Science Society of America Journal*, *66*(4), 1296-1303. https://doi.org/10.2136/sssaj2002.1296.

Simon, L. M., Obour, A. K., Holman, J. D., & Roozeboom, K. L. (2022). Long-term cover crop management effects on soil properties in dryland cropping systems. *Agriculture, Ecosystems & Environment*, *328*, 107852. https://doi.org/10.1016/j.agee.2022.107852.

Six, J., & Paustian, K. (2014). Aggregate-associated soil organic matter as an ecosystem property and a measurement tool. *Soil Biology and Biochemistry*, *68*, A4-A9. https://doi.org/10.1016/j.soilbio.2013.06.014.

Smika, D. E. (1990). Fallow management practices for wheat production in the Central Great Plains. *Agronomy Journal*, *82*(2), 319-323. https://doi.org/10.2134/agronj1990.00021962008200020029x.

Snapp, S.S., Swinton, S.M., Labarta, R., Mutch, D., Black, J.R., Leep, R., Nyiraneza, J., & O'Neil, K. (2005). Evaluating cover crops for benefits, costs and performance within cropping system niches. *Agronomy journal*, *97*(1), 322-332. https://doi.org/10.2134/agronj2005.0322a.

Soil Survey Staff. (2020). *Web Soil Survey.* U.S. Department of Agriculture, National Resources Conservation Service. http://www.websoilsurvey.nrcs.usda.gov

Thompson, N. M., Armstrong, S. D., Roth, R. T., Ruffatti, M. D., & Reeling, C. J. (2020). Short-run net returns to a cereal rye cover crop mix in a Midwest corn–soybean rotation. *Agronomy Journal*, *112*(2), 1068-1083. https://doi.org/10.1002/agj2.20132.

U.S. Bureau of Labor Statistics. (2022). *Producer Price Index by Commodity: All Commodities*. FRED, Federal Reserve Bank of St. Louis. https://fred.stlouisfed.org/series/PPIACO

Unger, P. W., & Vigil, M. F. (1998). Cover crop effects on soil water relationships. *Journal of soil and water conservation*, *53*(3), 200-207.

Van Bavel, C. H. M. (1950). Mean weight-diameter of soil aggregates as a statistical index of aggregation. *Proceedings. Soil Science Society of America, 1949*, *14*, 20-23. https://doi.org/10.2136/ sssaj1950.036159950014000C0005x.

Venter, Z. S., Jacobs, K., & Hawkins, H. J. (2016). The impact of crop rotation on soil microbial diversity: A meta-analysis. *Pedobiologia*, *59*(4), 215-223. https://doi.org/10.1016/j.pedobi.2016.04.001.

Wallander, S., Smith, D., Bowman, M., & Claassen, R. (2021). *Cover crop trends, programs, and practices in the United States* (No. 1476-2021-709). U.S. Department of Agriculture, Economic Research Service. https://www.ers.usda.gov/webdocs/publications/100551/eib-222.pdf?v=9246

Widman, N. (2004). *RUSLE2-Instructions and Users Guide*. U.S. Department of Agriculture, Natural Resources Conservation Service, Washington, DC.

Wittwer, R.A., Bender, S.F., Hartman, K., Hydbom, S., Lima, R.A., Loaiza, V., Nemecek, T., Oehl, F., Olsson, P.A., Petchey, O., Prechsl, U.E., Schlaeppi, K., Scholten, T., Seitz, S., Six, J., van der Heijden, M.G. (2021). Organic and conservation agriculture promote ecosystem multifunctionality. *Science advances*, *7*(34), eabg6995. https://doi.org/10.1126/sciadv.abg6995.

Xiong, C., He, J.Z., Singh, B.K., Zhu, Y.G., Wang, J.T., Li, P.P., Zhang, Q.B., Han, L.L., Shen, J.P., Ge, A.H., Wu, C.F., & Zhang, L.M. (2021a). Rare taxa maintain the stability of crop mycobiomes and ecosystem functions. *Environmental Microbiology*, *23*(4), 1907-1924. https://doi.org/10.1111/1462-2920.15262.

Xiong, C., Zhu, Y. G., Wang, J. T., Singh, B., Han, L. L., Shen, J. P., Li, P. P., Wang, G. B., Wu, C. F., Ge, A. H., Zhang, L. M., & He, J. Z. (2021b). Host selection shapes crop microbiome assembly and network complexity. *New Phytologist*, *229*(2), 1091-1104. https://doi.org/10.1111/nph.16890.

Zook, L. L. (1950). *Crop rotation and tillage experiments at the North Platte (Nebr.) Substation 1907-34* (No. 1488-2016-124653). U.S. Department of Agriculture, National Resources Conservation Service, Washington, DC. https://doi.org/10.22004/ag.econ.156272.

CHAPTER 5: COVER CROP FUNCTIONAL COMPOSITION IMPACTS ON BIOMASS PRODUCTION AND MICROBIALLY-MEDIATED ECOSYSTEM SERVICES

5.1. Introduction

Agricultural systems can provide many ecosystem services (ES), or environmental functions which are beneficial to humans, beyond food production (Millennium Ecosystem Assessment, 2005; Bennett & Balvanera, 2007; Tamburini et al., 2020). The integration of cover crops (CC) into crop rotations has been promoted as an option to support a range of ES, including improved nutrient cycling, C storage, and biodiversity conservation (Schipanski et al., 2014; Daryanto et al., 2018). The ES benefits associated with CC can be highly dependent the type of CC planted and associated traits. For example, grasses (Poaceae) have been shown to improved N retention due to high C:N ratio and competitive biomass production, while the N-fixation capability of legumes (Fabaceae) can contribute to increased N availability for subsequent cash crops (Finney, White & Kaye, 2016). Meanwhile, CC from the Brassicaceae family are commonly used for weed and pest suppression and to break up soil compaction (Wick et al., 2017), and deep, fibrous root systems of Asteraceae CC can cycle nutrients from soil layers inaccessible to other plant types (Dardanelli et al., 1997). These CC characteristics can be utilized to group species into functional types, which can be strategically selected to achieve specific management goals (Chapagain, Lee & Raizada, 2020; Kaye et al., 2019).

Beyond the selection of different functional groups, the diversity of CC mixtures can also influence the suite of ES provided. Ecosystem services including biomass production, maintenance of soil structure, and soil C sequestration have been shown to improve with greater CC diversity (Saleem et al., 2020). Diverse CC mixtures can also mitigate trade-offs among individual ES and contribute to greater agroecosystem multifunctionality (Finney & Kaye, 2016; Gross et al., 2017). For example, a study by Finney et al. (2017) showed that biomass production, weed suppression and N retention were bundled and negatively correlated with plant-available N and subsequent cash crop yields. While more diverse mixtures did not outperform monocultures in any individual ES, multifunctionality was improved by dampening ES trade-offs.

Many ES associated with CC, such as nutrient cycling, pathogen resistance and soil structure, are mediated by soil biological communities (Lehman et al, 2015a; Delgado-Baquerizo et al., 2020). Microbes in the rhizosphere have a particularly strong influence on plant and soil health by playing key roles in the suppression of plant pathogens, N fixation, metal chelation, among others (Jiao et al., 2021; Singh et al., 2020; Trivedi et al., 2020). Bacterial community composition has been shown to impact extracellular enzyme activity (EEA), which drives soil N processes and C turnover rates (Zheng et al., 2019; Schnecker et al., 2015; Trivedi et al., 2017). Understanding the role of microbial structure and function in ES provisioning is therefore gaining interest, as managing for certain microbial characteristics has potential to improve multiple agroecosystem functions (Lehman et al., 2015b).

Cover crop mixture selection may offer an avenue for intentional microbiome management as different species or functional types have been shown to favor the presence of certain microbial groups and influence overall microbial community composition (Finney, Buyer & Kaye, 2017; Martinez-Garcia et al., 2018; Kim et al., 2020; Muhammad et al., 2021). Microbial communities are recruited through differences in quality and quantity of plant residues and exudates, which can vary substantially among CC functional types (Seitz et al., 2022; Kramer et al., 2012). Legumes, for example, have been associated with increased abundance of actinomycetes and Gram-positive bacteria, while oat and forage radish have been associated with protozoa and Gram-negative bacteria (Finney et al., 2017; Muhammad et al., 2021). The extent to which mixture diversity impacts these species-specific associations is a topic of debate; some studies have reported microbial communities associated with mixtures which retain characteristics of their component species (Finney et al., 2017; Qiao et al., 2012), while others demonstrate that plant-microbe associations can be significantly altered by the presence of other species through changes in soil properties and diverse interactions among microbial groups (Bakker et al., 2013; Schlatter et al., 2015).

As CC species selection can impact both microbial community composition and the ES they regulate, CC offer a useful opportunity to elucidate the role of microbial communities in ES provision, while providing practical understanding of how different plant species can be strategically selected to achieve management goals. This study examined the effects of CC functional groups and diversity on biomass production, microbially-mediated ES, and microbial community structure. Microbially-mediated ES included C-, N- and P-cycling, soil aggregation, C sequestration, and conservation of belowground biodiversity. We expected individual CC functional types to differentially contribute to ES provisioning and that these ES benefits would be associated with distinct microbial communities. We also hypothesized that more diverse CC mixtures would conserve ES benefits and microbial associations of individual CC types, thereby maximizing multifunctionality. This research sought to inform the selection of CC species to improve agroecosystem function, while more broadly advancing our understanding of the role of microbial community composition in the provisioning of ES.

5.2 Methods

5.2.1 Experimental design

This research was conducted in the greenhouse at the Colorado State University Plant Growth Facilities in Fort Collins, CO. Fifteen CC treatments were established in a greenhouse experiment ranging in diversity from one to four functional types, with all factorial combinations of functional types included. Functional type was defined by including CC species from each of four plant families with distinct functional traits: Poaceae, Fabaceae, Brassicaceae, and Asteraceae. Functional types were always represented by two CC species to produce the average effect of more than one species within each functional type. Thus, each treatment had between two and eight plant species (Table 5.1). The CC species were selected to represent variations in nutrient-acquisition strategy (N_2-fixing vs. N-scavenging), temporal growth patterns (warm vs. cool season) and physiological classification (monocot vs. dicot) (Table 5.1). The experiment followed a randomized complete block design with each treatment present in five replicate blocks for a total of 75 experimental units.

5.2.2 Plant growth conditions

Soil was collected to a depth of 10 cm from the Southwestern Colorado Research Center in Yellow Jacket, CO (37°32' N latitude; 108°44' W longitude), where research is being conducted on long-term effects of CC on soil health and economic outcomes (see previous chapters 3 & 4). Soil at the research center is classified as a Wetherill loam (fine-silty, mesic Aridic Haplustalfs) (Soil Survey Staff, 2020) with a low soil organic C content (~0.8%) and neutral pH (6.9). Soil was air-dried, ground to pass through a 2 mm sieve, and mixed with sand prior to treatment establishment.

Pots (3.8 L) were filled with a soil:sand mixture (2:3 ratio by volume, to ensure drainage) and packed down to a bulk density of approximately 1.2 g/cm^3 prior to seeding. Seeds were sown into pots at a rate of two seeds more than the target seeding rate per species and thinned to the target rate two weeks after planting. The target seeding rate was set to approximate field seeding rates for monocultures of each species, with some adjustment for greenhouse conditions (Table S5.1).

To ensure consistent moisture levels among treatments, each pot was weighed at the start of the experiment after saturating the soil and allowing pots to drain for 24 hours, to determine approximate field capacity. Pots were watered every two days and brought up to the field capacity weight every four days. The week before harvest, target soil moisture was decreased to 50% of field capacity for ease of harvest. Pots were weeded weekly by hand. No fertilizer or pesticides were added.

5.2.3 Study harvest

Plant biomass and soils were sampled and analyzed to characterize microbial community composition and to evaluate ES including biomass production, C-, N- and P-cycling, maintenance of soil structure, maintenance of soil fertility, and conservation of belowground biodiversity (Table 5.2). Pots in all treatments were destructively harvested 62 days after planting, when the first species (winter pea) reached the flowering stage. This is common practice in the field to reduce potential for CC to go to seed and create weed issues in subsequent cash crops. Aboveground biomass was harvested by cutting plants at the soil surface and separating by species. This material was dried at 60°C and weighed. The remaining soil and root biomass was emptied onto a sterilized pan. Roots, rhizosphere soil, and bulk soil were then separated by shaking the main root ball three times to shed loose soil. Remaining soil adhering to the roots was considered to be rhizosphere soil, which was separated from the roots using sterilized tweezers and passed through a sterilized, 2 mm sieve. Rhizosphere soil was then homogenized and approximately 50 g were refrigerated for subsequent analyses. Bulk soil was passed through an 8 mm sieve, carefully picking out fine roots and breaking soil along natural planes of weakness and air-dried for analysis of aggregate stability via wet-sieving. All roots collected were placed on a 1 mm sieve, rinsed with water, and dried at 60°C to determine belowground biomass for each pot.

5.2.4 Soil analyses

Air-dried, 8 mm-sieved bulk soil was analyzed for wet aggregate stability according to methods adapted from Elliott (1986). A 40 g sample was submerged in deionized water for 5 minutes of slaking, and then progressively passed through a 2 mm, 250 µm, and 53 µm sieve by wet-sieving (50 oscillations over a 2 min period). Each aggregate fraction was rinsed into an aluminum pan and dried at 60°C. Aggregate stability is presented as mean weight diameter, which was calculated by multiplying the mass of each fraction by the average diameter of the corresponding fraction (van Bavel, 1950). Aggregate stability analysis was only performed on four of the five replicated blocks due to harvest issues with the first block.

A subsample of bulk soil was passed through a 2 mm sieve and further analyzed for permanganate-oxidizable C (POXC), or biologically active soil C, using methods detailed in Weil et al. (2003). Briefly, 2.5 g of air-dried soil was shaken in a 0.02 M solution of $KMnO_4$ and allowed to settle in a dark environment for 10 min. The diluted supernatant was read on a spectrophotometer for determination of POXC content.

Microbial community function was assessed by measuring activity of six extracellular enzymes including: C-acquisition enzymes [β-glucosidase (BG) and β-cellobiosidase (CB)], N-acquisition enzymes [β-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), tyrosine aminopeptidase (TAP)], and P-cycling enzymes [phosphatase (PHOS)]. Enzyme activity was measured by a fluorometric method in which two fluorescent indicators (4-methylumbelliferone and 7-amino-4-methylcoumarin) are released during an enzyme-catalyzed reaction (Bell et al., 2013). One day after harvest, approximately 1 g of refrigerated rhizosphere soil was mixed with a 50 mM Tris buffer and pipetted into deep 96-well plates for enzyme activity measurement and standard curves. Substrates were added and plates were covered and incubated at 25°C for approximately 4 hours to mimic average soil temperature. Fluorescence was measured on a microplate reader (Biotek Instruments). Activities were expressed as nmol h^{-1} g^{-1} dry soil. The remainder of the rhizosphere soil was frozen for future DNA extraction and analysis of microbial community structure. Microbial community function was only assessed on four of the five replicated blocks due to handling error during the harvest of the first replicated block.

5.2.5 Molecular soil microbial community analysis

Soil DNA was extracted using the Qiagen Powersoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA, USA) from 0.50 g of rhizosphere soil from each experimental unit. Primer sets 515F/806R (Caporaso et al., 2012) were used to amplify a portion of the bacterial 16S rRNA gene. Bioinformatics processing was performed using a combination of USEARCH (Edgar, 2010) and UNOISE3 (Edgar, 2016), and Amplicon Sequence Variant (ASV) tables were generated based on 97% sequence similarity. FastQC (Andrews, 2010) was used to assess sequencing run quality and sequences less than 100 bp, with a low-quality score ($Q < 20$), or with ambiguous nucleotides were discarded. Primers and adapters were removed using cutadapt (Martin, 2011). Samples were demultiplexed by merging paired-end reads and performing an initial quality check test. The UCLUST and UPARSE algorithm (Edgar, 2013) was then used to create a representative set database. To generate ASV tables at the sample level, unique ASVs were clustered using

DADA2, DeNoised using uNoise3 (Xiong et al., 2021a, 2021b) and mapped to the Silva database (Pruesse et al., 2007). Bacterial sequences that match host mitochondria and chloroplast were removed.

Tables of ASV abundance were rarefied to the minimum count per sample (7797 reads), excluding two samples with fewer than 1000 reads which were discarded. Based on the rarefaction curves, our rarified sequencing depth was appropriate to capture most of the diversity present in the samples. Alpha diversity (Shannon index), richness (ASV count), and evenness (Pielou's) for each sample were then quantified using the phyloseq package in R (McMurdie & Holmes, 2013). Rare phyla with <0.001% of total reads were removed and data were normalized using cumulative-sum squaring method (Paulson et al., 2013). Rarefication and normalization were done using the *phyloseq* and *metagenomeSeq* packages in R (McMurdie & Holmes, 2013; Paulson et al., 2013). As an indicator of community resource requirements, the ratio of oligotrophic:copiotrophic bacteria was calculated as the ratio of the sum of normalized abundances of Acidobacteria and Verrucomicrobia divided by the sum of normalized abundances of Actinobacteria, Betaproteobacteria and Firmicutes (Collins et al., 2016; Fierer, Bradford & Jackson, 2007; Trivedi et al., 2013).

5.2.6 Calculating relative yield total

Relative yield total (RYT) is a measure of overyielding that is used to determine whether mixtures are more productive than the average of their respective monocultures. Relative yield (RY) components were calculated for each functional type within each sample using the following equation:

$$RY = \frac{P_x}{M_x}$$

where P_x is the aboveground dry biomass yield of each component, x, when grown in polyculture (i.e., with multiple functional types: Poaceae, Fabaceae, Brassicaceae or Asteraceae), and M_x is the yield of each functional type grown alone. P_x within each replicate pot was divided by the M_x averaged across all blocks. Relative yield total was calculated for each experimental unit as the sum of all RY components present within the mixture.

5.2.7 Calculating multifunctionality scores

To assess multifunctionality and visualize trade-offs among ES, all proxies for ES (Table 5.2) were converted to a standardized unit ranging from 0.1 to 1 using the following homothetic transformation equation:

$$Y_i = 0.1 + \left(\frac{x_i - b_i}{a_i - b_i}\right)$$

where Y is the transformed value of the ES *i, x* is the measured indicator value, and *a* and *b* are the maximum and minimum observed values of the ES *i* across all pots, respectively (Kearney et al., 2019). The standardized score for each ES was either the standardized score of the proxy or, in cases where ES is associated with multiple proxies, the average of the proxies was used (see Table 5.2). Trade-offs among ES were visualized by performing a principal components analysis (PCA) on standardized scores. Multifunctionality scores were calculated for each experimental unit as the average of standardized values for all ES (Finney & Kaye, 2016).

5.2.8 Statistical analysis of ecosystem services

Cover crop functional type effects on ES proxies, multifunctionality, and microbial community metrics were analyzed using separate multifactor analysis of variance (ANOVA) with presence/absence of each functional type (coded as a binary variable) as fixed effects and block as a random effect. In the analysis of CC impacts on productivity (aboveground biomass, belowground biomass, total biomass, and RYT), all two-way interactions between functional types were also included to understand competition or complementarity among CC types, which could influence the relative contribution of individual CC types to ES provision in mixtures of greater CC diversity. Treatments with only one functional type were not included in the analysis of RYT. Post-hoc pairwise comparisons among treatments were estimated using Tukey-adjusted pairwise comparisons in the *emmeans* package (Lenth, 2018).

The effect of CC functional diversity was separately analyzed on each outcome variable using linear mixed effect models with functional diversity as a continuous predictor and block as a random effect. Due to uneven treatment distribution among levels of functional diversity, analysis was done using the *nlme* package in R to allow for heterogeneity of variance (Pinheiro & Bates, 2022).

5.2.9 Microbial community analysis

Treatment and CC functional type effects on microbial community composition were detected using PERMANOVA of Bray-Curtis dissimilarities of normalized taxon abundances. Canonical analysis of principal coordinates (CAP) was used, constrained by treatment. Differential abundance analysis was performed using the Analysis of Compositions of Microbiome with Bias Correction (ANCOM-BC) method at the class level to identify taxa which are significantly correlated (FDR-adjusted q-value<0.05) with CC functional types (Lin & Peddada, 2020).

Distance-based redundancy analysis (db-RDA) was performed to assess relationships between microbial community composition at the class level and measured ES proxies using a Bray-Curtis dissimilarity matrix. Stepwise backwards selection was used to select best model fit. Taxa which had significant effects on community separation were displayed on ordination plot and color coded according to copiotrophic or oligotrophic classification (Fierer et al., 2007; Trivedi et al. 2013). Spearman correlations were calculated between ES proxies which were selected in the db-RDA and normalized taxa abundance and for class-level taxa which were >0.2% of total counts for all samples. All microbial community analysis was performed using *phyloseq, vegan,* and *ANCOMBC* packages in R (McMurdie & Holmes, 2014; Oksanen et al., 2017; Lin & Peddada, 2020).

5.3 Results

5.3.1 Biomass production

Increased CC functional diversity was not significantly correlated with productivity (p = 0.20; $R^2 = 0.042$; Table 5.3). More diverse mixtures had less variable biomass production but did not exceed biomass production of the most productive monoculture (Fig. 5.1). Fabaceae grown without other CC types was the most productive of all treatments, and the presence of Fabaceae in mixtures and as a monoculture significantly increased aboveground and total biomass estimates ($p < 0.001$; Table 5.3). The presence of Poaceae resulted in less aboveground biomass than Fabaceae, but significantly increased root biomass production ($p < 0.001$). Treatments which included Brassicaceae had reduced aboveground, root and total biomass. Brassicaceae also had an interactive effect with all other functional types, reducing root biomass in treatments with Poaceae and Asteraceae but not in treatments with Fabaceae. Asteraceae was moderately productive by itself but significantly reduced total mixture biomass in more diverse treatments (Table 5.3). Fabaceae contributed to overyielding (RYT > 1.0), as treatments with Fabaceae had a RYT significantly greater than 1.0 (1.11 ± 0.03), and treatments without Fabaceae had an average RYT less than 1.0 (0.94 ± 0.04; Table 5.3).

5.3.2 Soil parameters

Cover crop functional diversity was not significantly correlated with any ES proxy measured, except for a slight positive relationship with NAG activity (p = 0.047; R^2=0.056).

Instead, CC functional type was a greater driver of ecosystem function. Treatments with Fabaceae experienced significant improvements in aggregation, increasing MWD from 752 um to 763 um ($p = 0.003$; Table 5.4). No differences in POXC were detected among treatments. The presence of Fabaceae increased activity of C-cycling enzymes (CB, BG), N-cycling enzymes NAG and LAP, and the P-cycling enzyme PHOS (Table 5.4). Brassicaceae also had a positive effect on CB, BG, and NAG activity (Table 5.4). Asteraceae and Poaceae did not significantly affect aggregation, POXC or enzyme activity.

5.3.3 Microbial community composition

Prokaryotic community composition differed among treatments (*PERMANOVA*, $p = 0.001$). Presence or absence of Fabaceae, Poaceae, and Brassicaceae all affected community composition (*PERMANOVA*, $p < 0.01$). The CAP ordination plot shows that treatments with only one CC type clustered according to functional type, and microbial communities converged in more diverse treatments (Fig. 5.2). Poaceae and Brassicaceae showed greater microbial community diversity and evenness, while Fabaceae showed lower diversity and evenness ($p < 0.05$; Table 5.4).

Differential abundance analysis identified prokaryotic classes most responsible for treatment separation. Fabaceae were associated with increased abundance of copiotrophic taxa within the phyla Betaproteobacteria, Firmicutes, and Saccharibacteria and the oligotrophic phylum Bacteroidetes, and decreased abundance of taxa pertaining to oligotrophic phyla Acidobacteria, Chloroflexi, Deltaproteobacteria, Elusimicrobia, Parcubacteria, Planctomycetes, and Verrucomicrobia ($q<0.05$; Table 5.5). Fewer taxa were associated with other functional types; Brassicaceae increased abundance of classes within phyla Cyanobacteria and Gracilibacteria, and Poaceae increased taxa within Cyanobacteria and Fibrobacteres.

Increased abundance of copiotrophs in Fabaceae treatments was also reflected in the calculated oligotroph:copiotroph ratio, which was 0.697 in treatments containing Fabaceae compared to 0.722 in treatments without Fabaceae ($p < 0.001$; Table 5.4).

5.3.4 Multifunctionality and trade-offs among ecosystem services

The first two principal components explain 64.8% of variance in standardized indices of ES. The PCA biplot indicated trade-offs among ES, with biomass production and soil structure bundled and negatively associated with prokaryotic diversity and, to a weaker extent, active C (Fig. 5.3). Enzyme activity for all nutrients represented a third ES bundle that was positively associated with biomass and soil structure along PC1, but separated along PC2. Cover crop mixtures containing Fabaceae were positively associated with biomass production, soil structure and enzyme activity but negatively associated with prokaryotic diversity and active C (Fig. 5.3). Treatments with Poaceae were generally associated with active C and prokaryotic diversity. Treatments with Brassicaceae were similar but also were associated with increased enzyme activity.

Cover crop mixtures containing Fabaceae had higher multifunctionality scores, due to associated increases in biomass production, soil structure and enzyme activity. Cover crop functional diversity also was positively correlated with multifunctionality ($p = 0.045$; $R^2=0.095$; Fig. 5.4), as ES benefits of individual CC types were expressed in diverse treatments (Table S5.2).

5.3.5 Correlation between microbial taxa abundance and ecosystem services

Distance-based redundancy analysis demonstrated significant correlation between ES proxies and prokaryotic community structure at the class level (Fig. 5.5; $p = 0.001$; $R^2=0.26$; Adj. $R^2=0.28$), with the strongest effects on ordination structure from total biomass ($p = 0.001$), alpha diversity ($p = 0.001$), CB activity ($p = 0.028$), NAG activity ($p = 0.009$), LAP activity ($p = 0.043$), and BG activity ($p = 0.105$). Copiotrophic and oligotrophic taxa tend to cluster, with copiotrophic organisms generally correlated with total biomass production and NAG and BG activity. Oligotrophic taxa tend to be concentrated on the lower left, associated with LAP activity and alpha diversity.

Spearman correlations confirmed relationships between class-level taxa and ES proxies which were selected in the db-RDA analysis (Table 5.6). Generally, oligotrophic taxa from phyla Acidobacteria, Chloroflexi, Elusimicrobia, Parcubacteria, Planctomycetes and Verrucomicrobia were negatively correlated with enzyme activity. These taxa also tended to be negatively correlated with biomass production and aggregation, and positively correlated with diversity, though not in all cases. Conversely, copiotrophic taxa belonging to Bacteroidetes, Betaproteobacteria, Firmicutes and Saccharibacteria tended to be positively correlated with EEA, aggregation and biomass production, and negatively correlated with alpha diversity. There was a strong, negative correlation between oligotroph:copiotroph ratio and alpha diversity ($p < 0.001$; $R^2 = 0.22$). Taxa that are classified as neither oligotrophic nor copiotrophic demonstrated similar relationships, having either positive or negative correlations with EEA, biomass production and aggregation (Table 5.6).

5.4 Discussion

Cover crop functional types were associated with the provision and trade-offs of individual ES. Poaceae and Brassicaceae improved alpha diversity and richness, Fabaceae enhanced aggregation and biomass production, and Fabaceae and Brassicaceae led to high enzyme activity, a proxy for nutrient cycling. These ES were also correlated with microbial taxa, demonstrating the importance of microbial community composition in ES provisioning. In diverse CC mixtures, ES benefits and microbial community shifts associated with individual functional types were conserved, contributing to increased multifunctionality (Fig. 5.4; Table S5.2).

5.4.1 Effect of CC functional composition on biomass production

The effect of biodiversity on biomass production has been widely studied, and in natural systems, the relationship between biodiversity and productivity is generally positive (Hooper et al., 2005). However, in agricultural systems this relationship can be less pronounced, and polycultures do not always outperform monocultures which are best suited for environmental conditions (Finney & Kaye, 2016; Florence et al., 2019). This was observed in the present study, as there was no correlation between functional diversity and productivity, and treatments with only Fabaceae produced the greatest total biomass (Fig. 5.1; Table 5.3). Generally, Poaceae species are shown to outperform Fabaceae (Florence et al., 2019; Meza et al., 2022), but Fabaceae may have had a competitive advantage in this study due to low N-availability and consistent in the study soil. Still, these conditions are likely relevant for many field settings as N fertilization of CC is not a common practice due to additional costs.

Meanwhile, N-scavenging functional types showed lower productivity (Table 5.3). Asteraceae was moderately productive when planted by itself but contributed little to biomass production in more diverse mixtures (Table 5.3). This is consistent with studies showing that Asteraceae productivity can be highly sensitive to intercropping and low inorganic soil N (Ruhlemann & Schmidtke, 2015). Low representation of Asteraceae in more diverse CC mixtures also likely limited Asteraceae effects on ES and microbial community composition. Brassicaceae showed low productivity when grown by itself but was well-represented in more diverse mixtures. The allelopathic potential of Brassicaceae may have contributed to its competitive advantage; Brassicaceae CC are frequently used for weed suppression as they exude glucosinolates, which can be toxic to other plants (Haramoto & Gallandt, 2004; Jabran et al., 2015). These compounds may have limited biomass production of other CC types, as evidenced by interactive indicating reductions in aboveground biomass of Poaceae and Asteraceae grown in the presence of Brassicaceae (Table 5.3).

The inclusion of Fabaceae in more diverse mixtures also increased biomass production, which was likely due to both high Fabaceae biomass production as well as complementary N-acquisition strategies. Recent studies of CC diversity effects on biomass suggest that increases in productivity due to diversity is attributed to the selection effect, or a greater likelihood for a diverse mixture to contain a highly productive species (Finney & Kaye, 2016; Florence et al., 2019). In the present study, a higher RYT in mixtures containing Fabaceae and an interactive effect between Fabaceae and Poaceae (Table 5.3) suggest that greater biomass production in diverse mixtures containing Fabaceae could also be due to complementarity or facilitation. Such interactions between functional types with different N-acquisition strategies (i.e., N-scavenging vs. N_2-fixing) are often shown to increase productivity (Finney & Kaye, 2016; Hooper & Dukes, 2004). Also, N-scavenging species can deplete soil N pools, forcing Fabaceae to rely on N_2-fixation to meet nutrient demands (Fornara & Tilman, 2009). These N-additions can also benefit productivity of other CC types, when Fabaceae N becomes available through exudation, decaying of senescent roots, or by other pathways (Meza et al., 2022; Nyfeler et al., 2011).

5.4.2 Effect of CC functional type on belowground diversity and community structure

Cover crop functional composition resulted in distinct rhizosphere microbial communities and broadly supports the copiotroph/oligotroph hypothesis that substrate quality and quantity favors microbes of distinct lifestyle strategies (Fierer et al., 2007; Trivedi et al., 2013). Fabaceae treatments not only produced more biomass, increasing substrate quantity, but Fabaceae roots also have lower C:N ratios, which may have enhanced substrate quality (Li et al., 2020). Improved resource availability typically favors the growth of copiotrophic microbes, or those which are fast-growing and exploit more labile C sources (Fierer et al., 2007; Eilers et al., 2010). Finney, Buyer & Kaye (2017) observed increases in copiotrophs after increases in CC root exudation and fine root turnover, and a study by Fierer et al. (2012) observed greater abundance of known copiotrophic taxa with increasing N availability. These copiotrophs included taxa from the phyla Actinobacteria, Bacteroidetes, Beta-proteobacteria, which all increased in abundance in Fabaceae treatments (Table 5.5). Meanwhile, oligotrophic taxa are slower-growing, more stress-tolerant, and have thus been observed in resource-limited conditions (Fierer et al., 2007; Schmidt et al., 2018). These taxa, such as Actinobacteria. Elusimicrobia, and Delta-proteobacteria, decreased in abundance in Fabaceae treatments. Changes in these taxa abundance were reflected by a significant decrease in oligotroph:copiotroph ratio in Fabaceae treatments (Table 5.4).

Conversely, the ratio of oligotrophs to copiotrophs was slightly increased in mixtures containing Poaceae. Quality and quantity of substrates were likely lower in these treatments, as Poaceae are associated with higher C:N ratios and more recalcitrant C compounds (Li et al., 2020). These treatments produced moderate amounts of biomass without N additions (Table 5.3). Exacerbated N limitation in these conditions likely favored the growth of oligotrophic taxa (Table 5.4, 5.5).

Literature shows that copiotrophic dominance can have a negative correlation with prokaryotic diversity due to decreased evenness (Jangid et al., 2008), which is consistent with our findings (Table 5.4). Oligotroph:copiotroph ratio had a strong negative correlation with alpha diversity ($R^2=0.22$; $p < 0.001$) and increases in copiotrophs in Fabaceae treatments was attributed to an increase in abundant taxa (Beta-proteobacteria and Firmicutes; Table 5.5), decreasing evenness as well as alpha diversity and richness (Table 5.4).

Brassicaceae treatments were associated with increased alpha diversity, but not with changes in oligotroph:copiotroph ratio. Still, differential abundance analysis revealed an increase in abundance of Cyanobacteria, a known oligotrophic phylum, as well as Gracilibacteria, for which the functional role is less clear. Other studies have shown that members of the Brassicaceae family can alter bacterial community structure (Finney et al., 2017; Hollister et al., 2013); specifically, rapeseed was associated with increased abundance of Gram-positive bacteria (Mackie et al., 2014).

5.4.3 Cover crop functional composition impacts on microbially-mediated ES

Differences in nutrient availability as well as microbial community composition appeared to mediate CC functional composition effects on microbial-mediated ES, namely EEA and aggregation. Generally, the production of extracellular enzymes can be stimulated by increased availability of the associated nutrient-containing substrate; increased C- and N-cycling enzymes have been positively associated with increased C and N inputs, respectively (Allison & Vitousek, 2005; Sinsabaugh et al., 2008). P-cycling enzymes are dependent on P substrate availability but can also be limited by N requirements for the production of phosphatase (Curtright & Tiemann, 2021). Enzyme activity was higher in Fabaceae treatments for all C-, N- and P-cycling enzymes except TAP (Table 5.4). Fabaceae generally have higher N concentration in their plant tissue, which may alleviate microbial N limitations that can slow enzyme production. Other studies have shown that the incorporation of Fabaceae increases C-, N- and P-cycling enzyme activity relative to other plant types (Dinesh et al., 2006; Cui et al., 2015; Singh et al., 2018), especially in nutrient poor soils (Curtright & Tiemann, 2021). This suggests that Fabaceae can increase microbial activity associated with nutrient cycling and likely microbial biomass and metabolic rates more broadly (Hartman & Richardson, 2013).

Despite reducing biomass production, Brassicaceae increased EEA for C-cycling enzymes. Some N-cycling enzymes also showed increased activity (NAG and TAP; Table 5.4), but others were likely limited by N availability. A study comparing the effects of crop rotation on EEA similarly showed elevated C-cycling enzymes when mustard was incorporated into a cereal-based crop rotation but reported no change in N- and P-cycling enzymes (Singh et al., 2018). Increased EEA may be due to the allelopathic potential of Brassicaceae, as exudation of allelopathic compounds has been shown to stimulate microbial activity, but more research that explores this relationship in Brassicaceae species is needed (Jabran et al., 2015; Zuo et al., 2014).

Aggregation, a process largely driven by microbial activity and root C inputs (Bronick & Lal, 2005), was also improved in Fabaceae treatments. Fabaceae impacts on aggregation are mixed; some studies have shown greater aggregation benefits associated with Poaceae (Blanco-Canqui & Jasa, 2019; Fonte et al., 2019), while others show significant improvements to aggregation in Fabaceae-based cropping systems (Udom & Omovbude, 2019; Hazra et al., 2019; Singh et al., 2018). Conflicting evidence may be due to differences in biomass production and resource limitations at the study site, as C inputs and activity of microbes and soil fauna drive aggregate formation (Bronick & Lal, 2005; Velásquez et al., 2012). Improvements in aggregation observed here are likely influenced by both increased biomass production and microbial activity in Fabaceae treatments. CC mixture composition had no impact on active C, potentially due to the short-term nature of the study.

Differences in copiotrophic and oligotrophic taxa described above seemed to mediate CC functional composition effects on microbially-mediated ES, as evidenced by db-RDA and

Spearman correlations. Copiotrophs exhibit higher growth rates and have been positively correlated with EEA and increased respiration (Trivedi et al., 2017). Improved EEA and aggregation in Fabaceae treatments suggest that associated copiotroph-dominated communities had elevated microbial activity. Lowered alpha diversity observed in Fabaceae treatments has also been correlated with EEA (Zheng et al., 2019). Spearman correlations between class-level microbial taxa and ES confirmed these relationships, with copiotrophic taxa generally positively correlated with enzyme activity and aggregation and negatively correlated with diversity. Oligotrophic taxa demonstrated opposing relationships, generally negative correlations with enzyme activity and aggregation and positive correlations with biodiversity. Taxa with no known ecological classification also followed these general trends, demonstrating either oligotrophic (in the case of Chlorobi, Fibrobacteres, and Woesearchaeota) or copiotrophic (in the case of WCHB1-60) tendencies.

5.4.4 Functional diversity reduced ES trade-offs and demonstrated greater multifunctionality

Due to plant-nutrient-microbe relationships detailed above, trade-offs were observed among ES bundles, with total biomass, soil structure, and EEA largely bundled and negatively associated with prokaryotic diversity and active C (Fig. 5.3). Though functional diversity was not strongly correlated with any individual ES, functionally diverse CC mixtures had greater multifunctionality scores (Fig. 5.4; $p = 0.045$). Generally, a lack of interactive effects between functional diversity and ES benefits associated with individual functional types showed that benefits were conserved in more diverse mixtures, thereby alleviating ES trade-offs. For example, the mixture with all four functional types experienced relatively higher soil structure, microbial activity, and biomass production associated with Fabaceae, while maintaining alpha diversity associated with Poaceae and Brassicaceae (Table 5.4, S5.1). Furthermore, functional diversity provides insurance that the species best-suited for agroecological conditions will be provided in the mixture. In the present study, Fabaceae best supported the majority of ES, as their N-fixing properties and improved substrate quality enabled greater biomass production and increased microbial activity, which resulted in improved soil structure. Complementarity seemed to contribute to greater multifunctionality in diverse CC mixtures as well, as evidenced by overyielding.

Microbial communities associated with CC functional types also converged in more diverse treatments (Fig. 5.2), with oligotroph:copiotroph ratio similar to that of Fabaceae and alpha diversity similar to that of Poaceae (Table S5.2). The maintenance of microbial associations of CC functional types in more diverse mixtures has been observed in other studies (Finney et al., 2017). Ecosystem service benefits were also maintained in more diverse mixtures, supporting the role of associated microbial community composition in maintaining ES benefits.

5.5. Conclusions

Our study establishes a clear link between CC functional composition, microbial communities, and ES provisioning. The incorporation of Fabaceae into CC mixtures led to more copiotrophic microbial communities with increased activity, which could provide improved nutrient cycling and soil structure. Meanwhile, Poaceae and Brassicaceae improved belowground biodiversity, which could have important implications for ecosystem resilience. These microbe-function relationships support the oligotroph-copiotroph approach to classifying complex microbial communities and can be utilized to manage for microbial communities which promote targeted soil functions and/or serve as early indicators of changes in soil health.

Our findings also highlight the efficacy of Fabaceae in improving agroecosystem function. Cover crop mixtures containing Fabaceae experienced greater biomass production, soil structure and potential nutrient cycling, presumably due to N fixation capabilities and improved substrate quality, though this was not directly measured. These impacts may have been particularly pronounced due to nutrient-depleted study soils and lack of CC fertilization, and results are therefore most applicable to similar conditions.

Finally, though plant-specific impacts on ES are likely to vary depending on context, our study supports the idea that CC mixture diversity can provide insurance that well-suited species are incorporated in the mixture and ES trade-offs will be less pronounced. While more diverse mixtures may not surpass well-suited monocultures in individual ES, CC functional type impacts on both ES provisioning and microbial community are conserved in diverse mixtures. These findings complement existing scientific and field-based knowledge to inform CC mixture selection for farm-level management goals.

CHAPTER 5 TABLES AND FIGURES

Table 5.1. Cover crop (CC) species selected to represent four CC functional types (Poaceae, Fabaceae, Brassicaceae, and Asteraceae) in a greenhouse study investigating ES impacts of CC functional composition. Functional types were selected to represent varying nutrient acquisition strategies (N_2-fixing vs. N-scavenging), structural classification (monocot vs. dicot), and temporal growth patterns (warm vs. cool season).

CC Functional Type	Physiological Classification	N-acquisition strategy	Temporal growth pattern	CC Species Common Name	CC Species Scientific Name
Poaceae	Monocot	N-scavenging	Cool-season	Annual Ryegrass Triticale	*Lolium multiflorum* *x Triticosecale*
Fabaceae	Dicot	N_2-fixing	Cool-season	Common Vetch Winter Pea	*Vicia sativa* *Pisum sativum*
Brassicaceae	Dicot	N-scavenging	Cool-season	Rapeseed Forage Radish	*Brassica napus* *Raphanus sativus*
Asteraceae	Dicot	N-scavenging	Warm-season	Sunflower Safflower	*Helianthus annuus* *Carthamus tinctorius*

Table 5.2. Ecosystem services (ES) and associated proxies evaluated in in a greenhouse investigating ES impacts of CC functional composition.

ES	ES Proxy
Biomass production	Total aboveground and root biomass
Maintenance of soil structure	Wet aggregate stability
Maintenance of soil fertility	Permanganate-oxidizable C
C-Cycling	CB[1] enzyme activity
	BG[2] enzyme activity
N-Cycling	NAG[3] enzyme activity
	LAP[4] enzyme activity
	TAP[5] enzyme activity
P-Cycling	PHOS[6] enzyme activity
Conservation of belowground biodiversity	Prokaryotic alpha diversity
	Prokaryotic richness

[1] CB, β-cellobiosidase. [2] BG, β-glucosidase. [3] NAG, β-N-acetylglucosaminidase. [4] LAP, leucine aminopeptidase. [5] TAP, tyrosine aminopeptidase. [6] PHOS, phosphatase.

Table 5.3. Relative yield total (RYT) and total, root, and aboveground biomass for cover crop (CC) treatments of varying functional composition in a greenhouse study. Treatments included factorial combinations of CC functional types [Poaceae (P), Fabaceae (F), Brassicaceae (B), and Asteraceae (A)] with functional diversity (FD) varying between one and four types. Values represent the mean of each treatment (n = 5). Multifactor analysis of variance (ANOVA) was used to assess impacts of CC functional types. The impact of FD on each metric was separately analyzed using linear mixed models. Directionality of effect (+ or -) is shown along with significance (. $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

FD	CC Type	RYT	Total Biomass g pot^{-1}	Root Biomass g pot^{-1}	Aboveground Total g pot^{-1}	Aboveground Biomass by Functional Type			
						P g pot^{-1}	F g pot^{-1}	B g pot^{-1}	A g pot^{-1}
1	P	NA [1]	8.48	4.48	4.00	4.00	NA	NA	NA
	F	NA	12.05	1.64	10.41	NA	10.41	NA	NA
	B	NA	5.43	1.29	4.15	NA	NA	4.15	NA
	A	NA	5.85	1.06	4.79	NA	NA	NA	4.79
2	PF	1.044	10.37	3.48	6.90	2.47	4.42	NA	NA
	PB	0.922	5.76	1.99	3.77	1.60	NA	2.17	NA
	PA	1.001	6.89	2.63	4.26	2.72	NA	NA	1.54
	FA	1.103	10.14	1.52	8.62	NA	6.18	NA	2.44
	FB	1.123	9.53	1.84	7.69	NA	5.05	2.64	NA
	BA	0.938	5.13	1.14	3.99	NA	NA	3.25	0.74
3	PFA	1.175	9.97	2.94	7.03	2.67	3.57	NA	0.78
	PFB	1.018	8.57	2.70	5.87	1.18	2.81	1.88	NA
	PBA	0.932	6.02	2.10	3.93	1.27	NA	1.86	0.80
	FBA	1.200	9.46	2.11	7.35	NA	3.77	2.79	0.79
4	PFBA	1.115	9.54	2.76	6.78	1.26	3.49	1.25	0.78
			ANOVA						
	FD	NS [2]	NS	NS	NS				
	P	NS	NS	+ ***	- ***				
	F	+ ***	+ ***	NS	+ ***				
	B	NS	- ***	- **	- ***				
	A	NS	- *	- *	NS				
	P x F	NS	*	NS	**				
	P x B	NS	NS	**	**				
	P x A	NS	.	NS	**				
	F x B	NS	NS	*	NS				
	F x A	NS	NS	.	NS				
	B x A	.	**	**	*				

[1] NA, not applicable. [2] NS, not significant at alpha = 0.10.

Table 5.4. Analysis of cover crop (CC) functional composition impacts on microbial community metrics, ecosystem service proxies, and multifunctionality. Data are from a greenhouse study which included CC treatments with factorial combinations of CC functional types [Poaceae (P), Fabaceae (F), Brassicaceae (B), and Asteraceae (A)] and with functional diversity (FD) varying between one and four types. Multifactor analysis of variance (ANOVA) was used to assess impacts of CC functional types. The impact of FD on each metric was separately analyzed using linear mixed models. Directionality of effect (+ or -) is shown along with significance (. $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

CC Type	Soil Structure	Soil Fertility	C-Cycling		N-Cycling		P-Cycling		Microbial Community				MF Score
	MWD [1]	POXC [2]	CB [3]	BG [4]	NAG [5]	LAP [6]	TAP [7]	PHOS [8]	Shannon Diversity	Richness	Pielou's Evenness	Oligo: Copio [9]	
P	NS [10]	NS	NS	- .	NS	NS	NS	- .	+ ***	+ **	+ ***	+ .	NS
F	+ **	NS	+ *	+ *	+ **	+ **	NS	+ ***	- ***	- ***	- ***	- ***	+ **
B	NS	NS	+ ***	+ *	+ ***	NS	NS	NS	+ **	+ ***	+ .	NS	+ .
A	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FD	NS	NS	NS	NS	+ *	+ .	NS	NS	NS	NS	NS	- .	+ *

[1] MWD, mean weight diameter. [2] POXC, permanganate-oxidizable C. [3] CB, β-cellobiosidase. [4] BG, β-glucosidase. [5] NAG, β-N-acetylglucosaminidase. [6] LAP, leucine aminopeptidase. [7] TAP, tyrosine aminopeptidase. [8] PHOS, phosphatase. [9] Oligo:Copio, ratio of oligotrophic to copiotrophic microbes. [10] Not significant at an alpha level of 0.10.

Table 5.5. Summary of differential abundance at class level for distinct cover crop (CC) functional types. Data are from a greenhouse study which included CC treatments with factorial combinations of CC functional types [Poaceae (P), Fabaceae (F), Brassicaceae (B), and Asteraceae (A)] and with functional diversity (FD) varying between one and four types.

CC Type	Phylum	Class	Beta [1]	FDR-adjusted q-value [2]
Fabaceae				
	Bacteroidetes	Cytophagia	-0.038	0.001
	Pseudomonadota	Betaproteobacteria	0.034	0.023
	Firmicutes	Erysipelotrichia	0.487	<0.001
	Saccharibacteria	Saccharibacteria	0.702	<0.001
	Acidobacteria	Subgroup_22	-0.093	0.023
	Chloroflexi	Anaerolineae	-0.037	0.006
	Deltaproteobacteria	Deltaproteobacteria	-0.029	0.005
	Elusimicrobia	Elusimicrobia	-0.117	<0.001
	Parcubacteria	Parcubacteria	-0.076	0.001
	Planctomycetes	BD7-11	-0.111	0.005
	Planctomycetes	OM190	-0.121	0.001
	Planctomycetes	vadinHA49	-0.105	<0.001
	Verrucomicrobia	Verrucomicrobia	-0.200	0.002
	Aerophobetes	Aerophobetes	-0.253	0.046
	Candidate_division_OP3	Candidate_division_OP3	-0.317	0.041
	Nitrospirae	Nitrospira	-0.043	0.028
	Omnitrophica	NPL-UPA2	-0.224	0.004
	SM2F11	SM2F11	-0.082	<0.001
	WCHB1-60	WCHB1-60	0.476	<0.001
	Woesearchaeota	Woesearchaeota	-0.187	<0.001
Brassicaceae				
	Cyanobacteria	Cyanobacteria	0.444	0.010
	Gracilibacteria	Gracilibacteria	0.067	0.006
Poaceae				
	Cyanobacteria	Melainabacteria	0.050	0.001
	Fibrobacteres	Fibrobacteria	0.257	<0.001

[1] Analysis done using the Analysis of Compositions of Microbiome with Bias Correction method (Lin & Peddada, 2020). Positive values indicate a higher abundance in the presence of CC functional type. [2] Controls false discovery rate (FDR).

Table 5.6. Spearman correlation coefficients (ρ) relating rhizosphere class abundance of copiotrophic, oligotrophic, and non-classified prokaryotes with ecosystem service proxies. Data are from a greenhouse study with CC treatments of different functional compositions, and ecosystem service proxies relevant to microbial community composition were selected using distance-based Redundancy Analysis (Fig. 5.5). For simplicity, taxa displayed are only those that were significantly correlated with the activity of at least one enzyme. Positive and negative correlation coefficients are displayed in blue and red, respectively. Significance is also displayed (. $p < 0.10$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Classification	Phylum	Class	CB[7]	BG[8]	NAG[9]	LAP[10]	PHOS[11]	MWD[12]	DIV[13]	Total Plant Biomass
Copiotroph [1]	Bacteroidetes	Flavobacteria			0.30 *					-0.26 *
Copiotroph [1]	Bacteroidetes	Sphingobacteria							-0.46 ***	0.37 **
Copiotroph [1]	Pseudomonadota	Betaproteobacteria					0.39 **	0.39 **	-0.66 ***	0.44 ***
Copiotroph [2]	Firmicutes	Bacilli			-0.27 *				-0.52 ***	0.36 **
Copiotroph [2]	Gemmatimonadetes	Gemmatimonadetes								
Copiotroph [3]	Saccharibacteria	Saccharibacteria		0.26 *			0.35 **			0.58 ***
Oligotroph [1]	Acidobacteria	Acidobacteria			-0.26 *				-0.30 **	
Oligotroph [1]	Acidobacteria	Holophagae			-0.30 *					
Oligotroph [2]	Chloroflexi	Anaerolineae		-0.32 *	-0.38 **		-0.39 **	-0.34 *		
Oligotroph [2]	Chloroflexi	SHA-26	-0.31 *	-0.27 *		-0.29 *				
Oligotroph [2]	Cyanobacteria	ML635J-21				0.32 *			-0.35 **	
Oligotroph [4]	Elusimicrobia	Elusimicrobia					-0.27 *	-0.35 *	0.54 ***	-0.54 ***
Oligotroph [5]	Parcubacteria	Parcubacteria				-0.29 *			0.55 ***	-0.32 **
Oligotroph [2]	Planctomycetes	BD7-11		-0.31 *		-0.27 *	-0.43 **		0.37 **	-0.26 *
Oligotroph [2]	Planctomycetes	vadinHA49				-0.26 *			0.55 ***	-0.48 ***
Oligotroph [2]	Verrucomicrobia	Opitutae							0.27 *	
Oligotroph [2]	Verrucomicrobia	OPB35 soil group		-0.31 *	-0.26 *		-0.27 *			0.31 **
NC [6]	Aerophobetes	Aerophobetes			-0.30 *	-0.27 *	-0.36 **			
NC	Candidate division SR1	Candidate division SR1								
NC	Chlamydiae	Chlamydiae					0.27 *			
NC	Chlorobi	Chlorobia	-0.35 **		-0.38 **			-0.44 **		
NC	Fibrobacteres	Fibrobacteria	-0.27 *	-0.27 *	-0.30 *		-0.29 *		0.33 **	
NC	Latescibacteria	Latescibacteria		-0.29 *						
NC	Microgenomates	Microgenomates								
NC	WCHB1-60	WCHB1-60				0.30 *	0.31 *	0.32 *	-0.53 ***	0.65 ***
NC	Woesearchaeota	Woesearchaeota					-0.26 *	-0.43 **	0.43 ***	-0.61 ***

[1] Fierer et al. (2007); [2] Ho et al. (2017); [3] Wang et al. (2021); [4] Prescott et al. (2019); [5] Nelson & Stegen (2015); [6] Not Classified. [7] CB, β-cellobiosidase. [8] BG, β-glucosidase. [9] NAG, β-N-acetylglucosaminidase. [10] LAP, leucine aminopeptidase. [11] PHOS, phosphatase. [12] MWD, mean weight diameter. [13] DIV, prokaryotic diversity.

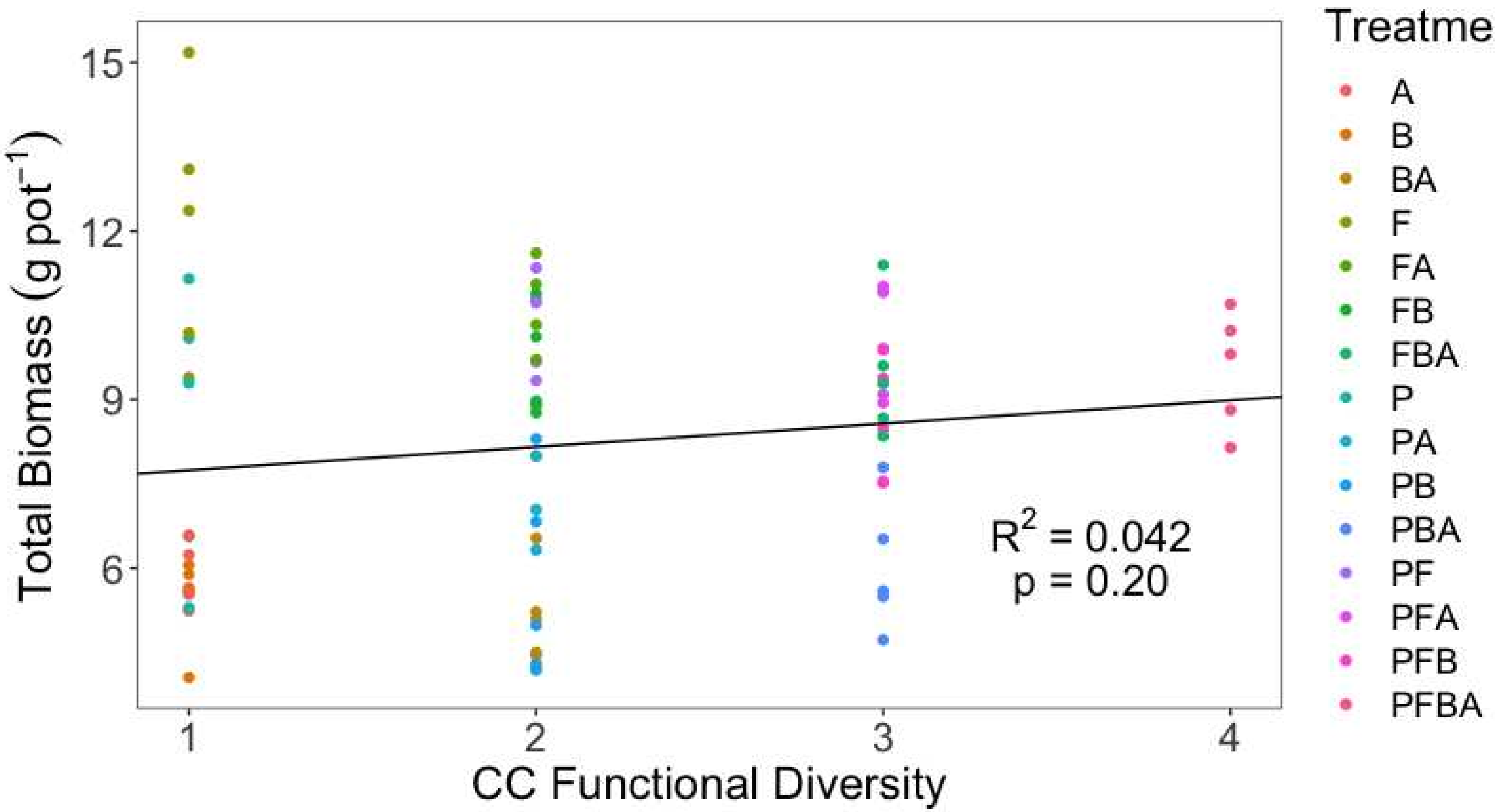

Figure 5.1. Correlation ($p = 0.20$; $R^2 = 0.042$) between cover crop (CC) functional diversity and total CC biomass among CC treatments with different functional compositions in a greenhouse study. Treatments included factorial combinations of CC functional types [Poaceae (P), Fabaceae (F), Brassicaceae (B), and Asteraceae (A)] with functional diversity (FD) varying between one and four types.

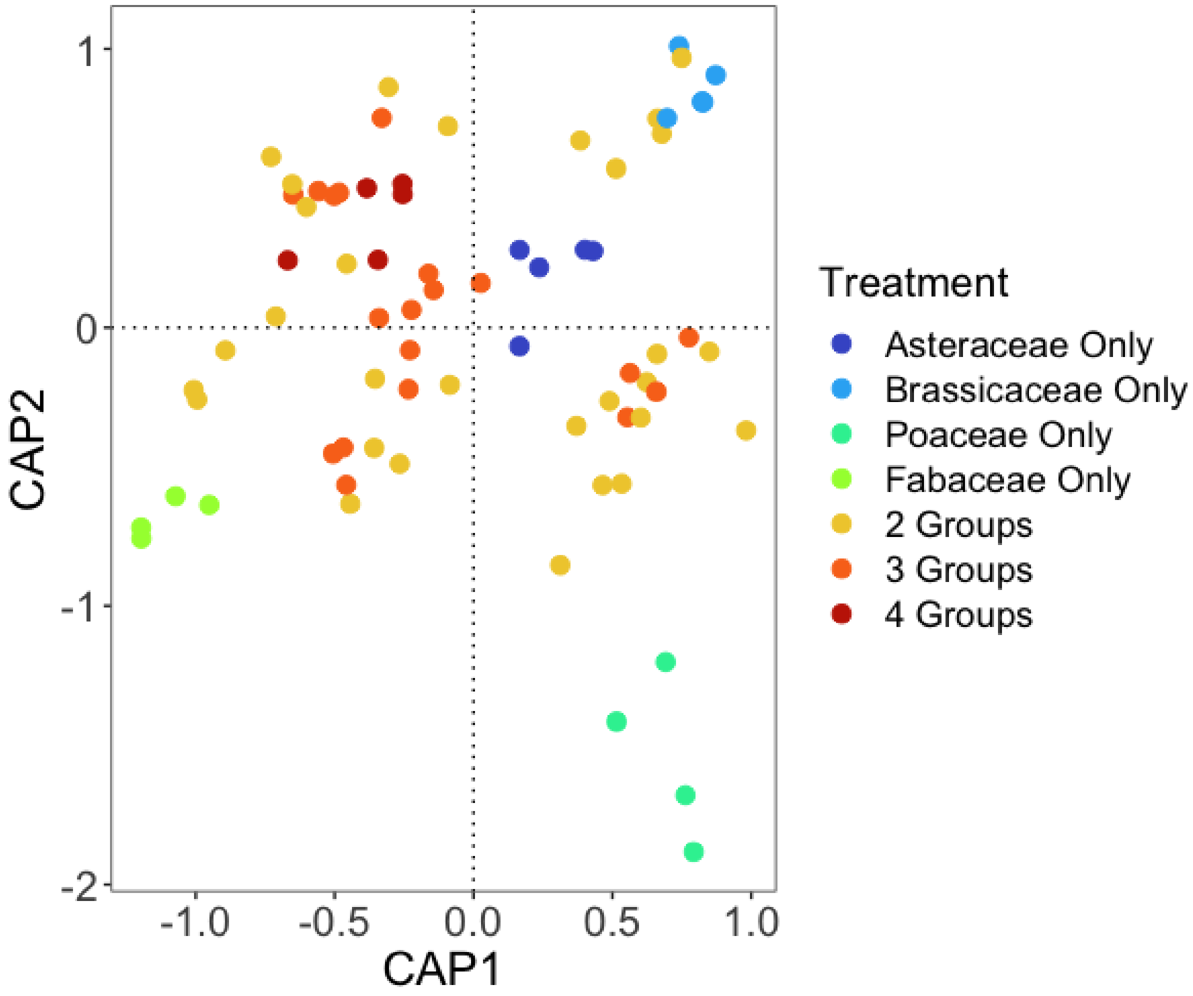

Figure 5.2. Canonical analysis of principal coordinates (CAP) of prokaryotic communities at the amplicon sequence variant (ASV) level constrained by cover crop (CC) treatment with distinct functional compositions. Data are from a greenhouse study which included 15 CC treatments with factorial combinations of CC functional types [Poaceae (P), Fabaceae (F), Brassicaceae (B), and Asteraceae (A)] and with functional diversity (FD) varying between one and four types. Permutational multivariate analysis of variance (PERMANOVA) indicated that there is a significant ($p < 0.001$) effect of CC treatment.

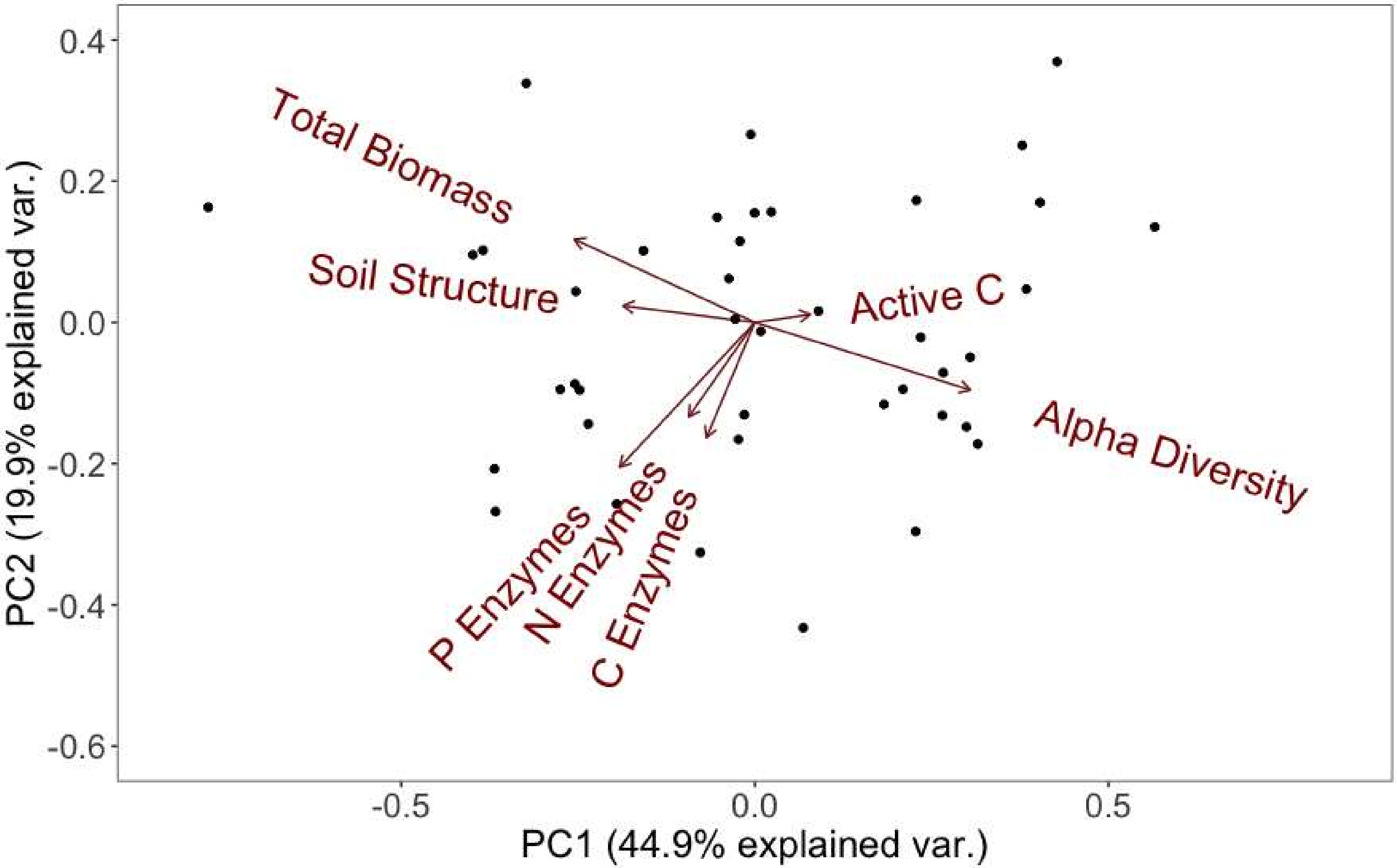

Figure 5.3. Principal component analysis (PCA) ordination of ecosystem services from a greenhouse study which included CC treatments with different functional compositions.

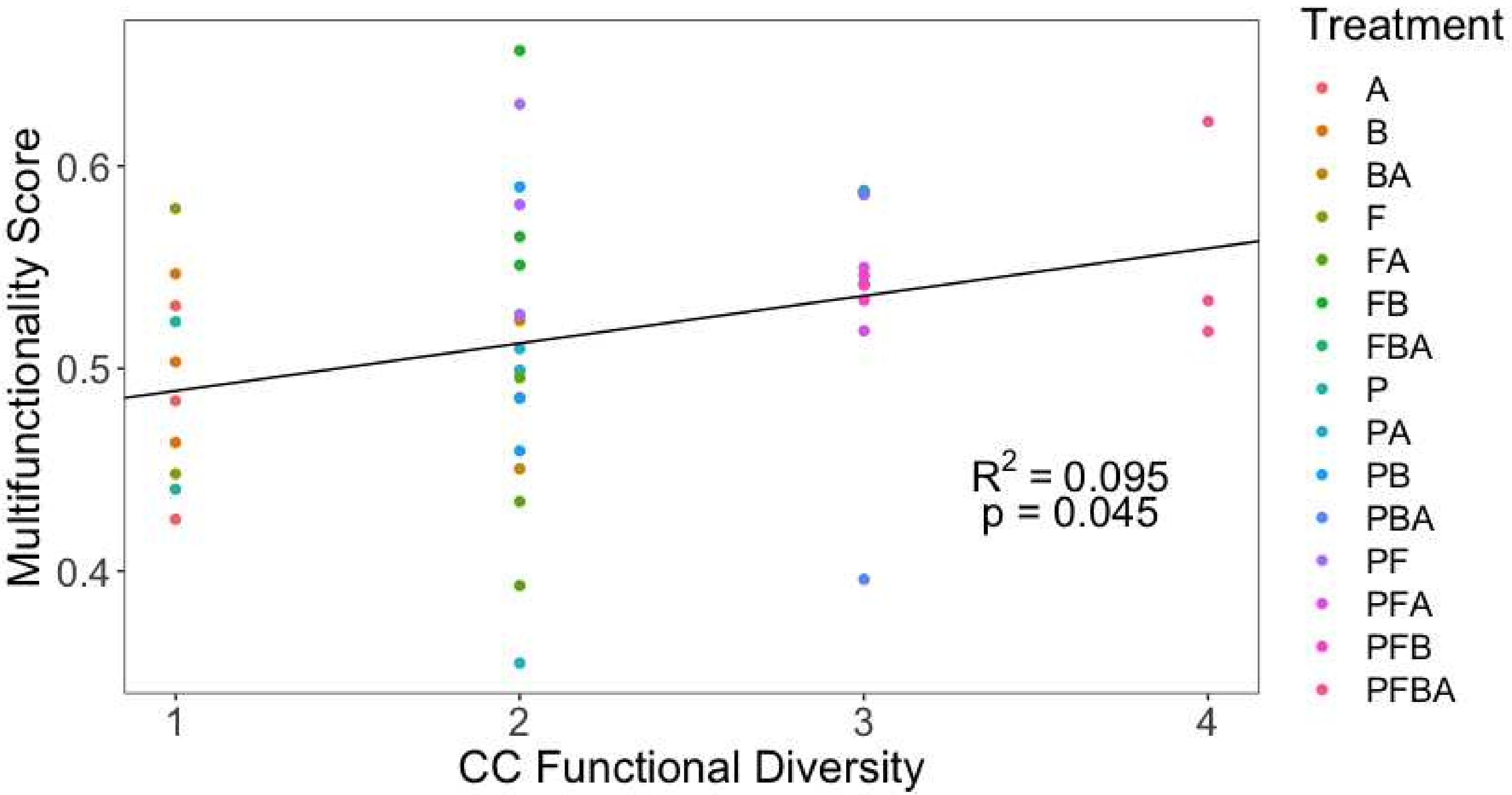

Figure 5.4. Correlation ($p = 0.045$; R^2=0.095) between cover crop (CC) functional diversity and multifunctionality scores among CC treatments with different functional compositions in a greenhouse study. Treatments included factorial combinations of CC functional types [Poaceae (P), Fabaceae (F), Brassicaceae (B), and Asteraceae (A)] with functional diversity (FD) varying between one and four types. Multifunctionality was measured by averaging standardized proxies for ecosystem functions.

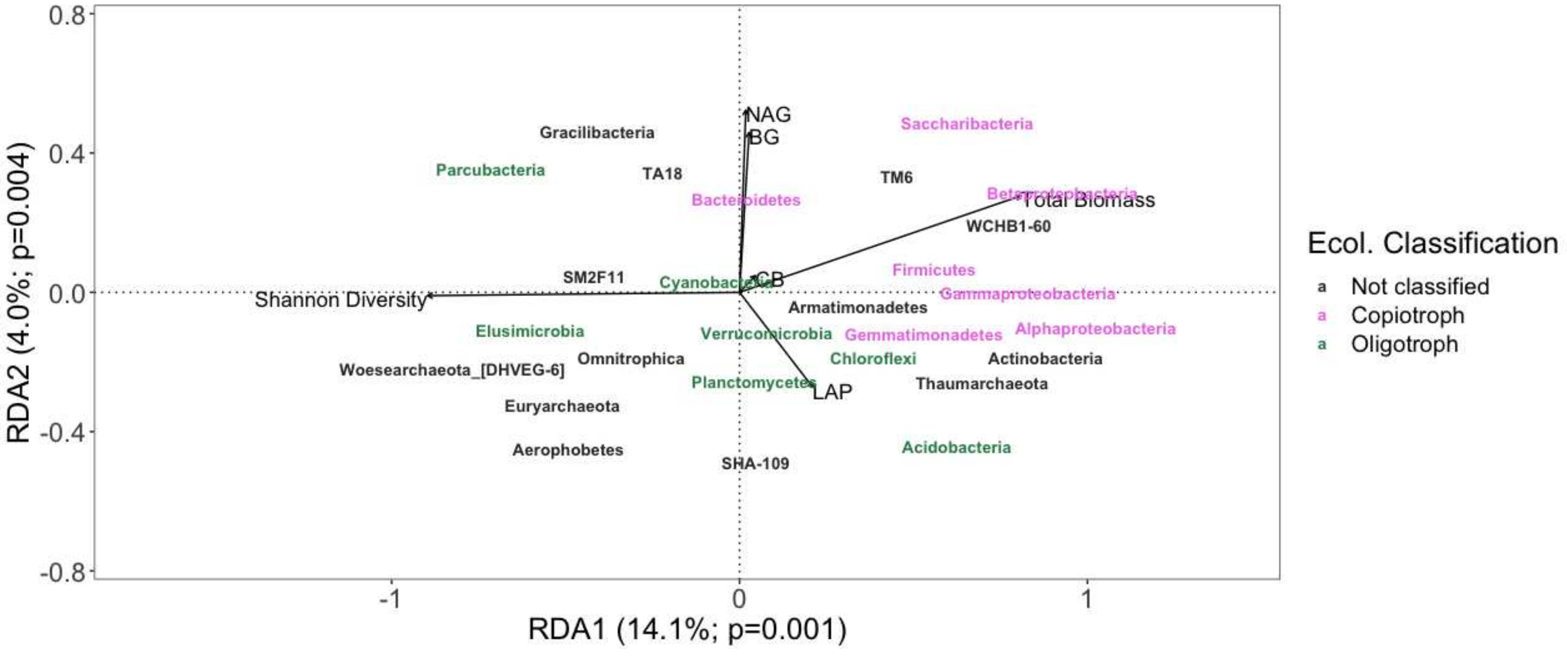

Figure 5.5. Distance-based Redundancy Analysis (db-RDA) relating prokaryotic community composition at the class level with ecosystem service proxies (p = 0.001; R^2=0.26; Adj. R^2=0.28). Bray-Curtis dissimilarities were used based on 16S rRNA sequencing. Backwards model selection determined variables with most explanatory power, including total biomass (p = 0.001), Shannon Diversity (p = 0.001), CB (β-cellobiosidase) activity (p = 0.028), NAG (β-N-acetylglucosaminidase) activity (p = 0.009), LAP (leucine aminopeptidase) activity (p = 0.043), and BG (β-glucosidase) activity (p = 0.105). Taxa which had significant effects on community separation (p < 0.01) are displayed on ordination plot and are color coded by copiotrophic or oligotrophic classification. Copiotrophic and oligotrophic classifications are supported by Fierer et al. (2007), Ho et al. (2017), Prescott et al., (2019), Padmanabhan et al., (2003), Nelson et al. (2015), and Wang et al. (2021).

REFERENCES

Allison, S. D., & Vitousek, P. M. (2005). Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology and Biochemistry*, *37*(5), 937-944. https://doi.org/10.1016/j.soilbio.2004.09.014

Andrews, S. (2010). FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Bakker, M. G., Bradeen, J. M., & Kinkel, L. L. (2013). Effects of plant host species and plant community richness on streptomycete community structure. *FEMS microbiology ecology*, *83*(3), 596-606. https://doi.org/10.1111/1574-6941.12017

Bell, C. W., Fricks, B. E., Rocca, J. D., Steinweg, J. M., McMahon, S. K., & Wallenstein, M. D. (2013). High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *Journal of Visualized Experiments*, *81*, e50961. https://doi.org/10.3791/50961

Bennett, E. M., & Balvanera, P. (2007). The future of production systems in a globalized world. *Frontiers in Ecology and the Environment*, *5*(4), 191-198. https://doi.org/10.1890/1540-9295(2007)5[191:TFOPSI]2.0.CO;2

Blanco-Canqui, H., & Jasa, P. J. (2019). Do grass and legume cover crops improve soil properties in the long term? *Soil Science Society of America Journal*, *83*(4), 1181-1187. https://doi.org/10.2136/sssaj2019.02.0055

Bronick, C. J., & Lal, R. (2005). Soil structure and management: A review. *Geoderma*, *124*(1–2), 3–22. https://doi.org/10.1016/j.geoderma.2004.03.005

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., ... & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and

MiSeq platforms. *The ISME journal*, *6*(8), 1621-1624. https://doi.org/10.1038/ismej.2012.8.

Chapagain, T., Lee, E. A., & Raizada, M. N. (2020). The potential of multi-species mixtures to diversify cover crop benefits. *Sustainability*, *12*(5), 2058. https://doi.org/10.3390/su12052058

Collins, C. G., Carey, C. J., Aronson, E. L., Kopp, C. W., & Diez, J. M. (2016). Direct and indirect effects of native range expansion on soil microbial community structure and function. *Journal of Ecology*, *104*(5), 1271-1283. https://doi.org/10.1111/1365-2745.12616

Cui, H., Zhou, Y., Gu, Z., Zhu, H., Fu, S., & Yao, Q. (2015). The combined effects of cover crops and symbiotic microbes on phosphatase gene and organic phosphorus hydrolysis in subtropical orchard soils. *Soil Biology and Biochemistry*, *82*, 119-126. https://doi.org/10.1016/j.soilbio.2015.01.003

Curtright, A. J., & Tiemann, L. K. (2021). Intercropping increases soil extracellular enzyme activity: a meta-analysis. *Agriculture, Ecosystems & Environment*, *319*, 107489. https://doi.org/10.1016/j.agee.2021.107489

Dardanelli, J. L., Bachmeier, O. A., Sereno, R., & Gil, R. (1997). Rooting depth and soil water extraction patterns of different crops in a silty loam Haplustoll. *Field Crops Research*, *54*(1), 29-38. https://doi.org/10.1016/S0378-4290(97)00017-8

Daryanto, S., Fu, B., Wang, L., Jacinthe, P., & Zhao, W. (2018). Quantitative synthesis on the ecosystem services of cover crops. *Earth Science Reviews*, *185*, 357–373. https://doi.org/10.1016/j.earscirev.2018.06.013

Delgado-Baquerizo, M., Reich, P. B., Trivedi, C., Eldridge, D. J., Abades, S., Alfaro, F. D., Bastida, F., Berhe, A. A., Cutler, N. A., Gallardo, A., García-Velázquez, L., Hart, S. C., Hayes, P. E., He, J. Z., Hseu, Z. Y., Hu, H. W., Kirchmair, M., Neuhauser, S., Perez, C. A., Reed, S. C., Santos, F., Sullivan, B. W., Trivedi, P., Wang, J. T., Weber-Grullon, L., Williams, M.A., & Singh, B. K. (2020). Multiple elements of soil biodiversity drive ecosystem functions across biomes. *Nature Ecology & Evolution*, *4*(2), 210-220. https://doi.org/10.1038/s41559-019-1084-y

Dinesh, R., Suryanarayana, M. A., Chaudhuri, S. G., Sheeja, T. E., & Shiva, K. N. (2006). Long-term effects of leguminous cover crops on biochemical and biological properties in the organic and mineral layers of soils of a coconut plantation. *European journal of soil biology*, *42*(3), 147-157.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics, 26*(19), 2460-2461. https://doi.org/10.1093/bioinformatics/btq461.

Edgar, R. C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature methods*, *10*(10), 996-998. https://doi.org/10.1038/nmeth.2604.

Edgar, R. C. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *BioRxiv*, 081257. https://doi.org/10.1101/081257.

Eilers, K. G., Lauber, C. L., Knight, R., & Fierer, N. (2010). Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biology and Biochemistry*, *42*(6), 896-903. https://doi.org/10.1016/j.soilbio.2010.02.003

Elliott, E. T. (1986). Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. *Soil science society of America journal*, *50*(3), 627-633. https://doi.org/10.2136/ sssaj1986.03615995005000030017x.

Fierer, N., Bradford, M. A., & Jackson, R. B. (2007). Toward an ecological classification of soil bacteria. *Ecology*, *88*(6), 1354-1364. https://doi.org/10.1890/05-1839

Fierer, N., Lauber, C. L., Ramirez, K. S., Zaneveld, J., Bradford, M. A., & Knight, R. (2012). Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME journal*, *6*(5), 1007-1017. https://doi.org/10.1038/ismej.2011.159

Finney, D. M., Buyer, J. S., & Kaye, J. P. (2017). *Living cover crops have immediate impacts on soil microbial community structure and function*. *72*(4), 361–373. https://doi.org/10.2489/jswc.72.4.361

Finney, D. M., & Kaye, J. P. (2016). Functional diversity in cover crop polycultures increases multifunctionality of an agricultural system. *Journal of Applied Ecology*. https://doi.org/10.1111/1365-2664.12765

Finney, D. M., Murrell, E. G., White, C. M., Baraibar, B., Barbercheck, M. E., Bradley, B. A., Cornelisse, S., Hunter, M. C., Kaye, J. P., Mortensen, D. A., Mullen, C. A., & Schipanski, M. E. (2017). *Ecosystem Services and Disservices Are Bundled in Simple and Diverse Cover Cropping Systems*. 1–5. https://doi.org/10.2134/ael2017.09.0033

Finney, D. M., White, C. M., & Kaye, J. P. (2016). Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. *Agronomy Journal*, *108*(1), 39-52. https://doi.org/10.2134/agronj15.0182.

Florence, A. M., Higley, L. G., Drijber, R. A., Francis, C. A., & Lindquist, J. L. (2019). Cover crop mixture diversity, biomass productivity, weed suppression, and stability. *PLoS one*, *14*(3), e0206195. https://doi.org/10.1371/journal.pone.0206195

Fonte, S. J., Botero, C., Quintero, D. C., Lavelle, P., & van Kessel, C. (2019). Earthworms regulate plant productivity and the efficacy of soil fertility amendments in acid soils of the Colombian Llanos. *Soil Biology and Biochemistry*, *129*, 136-143. https://doi.org/10.1016/j.soilbio.2018.11.016

Fornara, D. A., & Tilman, D. (2009). Ecological mechanisms associated with the positive diversity–productivity relationship in an N-limited grassland. *Ecology*, *90*(2), 408-418. https://doi.org/10.1890/08-0325.1

Gross, N., Bagousse-Pinguet, Y. L., Liancourt, P., Berdugo, M., Gotelli, N. J., & Maestre, F. T. (2017). Functional trait diversity maximizes ecosystem multifunctionality. *Nature Ecology & Evolution*, *1*(5), 1-9. https://doi.org/10.1038/s41559-017-0132

Haramoto, E. R., & Gallandt, E. R. (2004). Brassica cover cropping for weed management: A review. *Renewable agriculture and food systems*, *19*(4), 187-198. https://doi.org/10.1079/RAFS200490

Hartman, W. H., & Richardson, C. J. (2013). Differential nutrient limitation of soil microbial biomass and metabolic quotients (qCO_2): is there a biological stoichiometry of soil microbes? *PLoS one*, *8*(3), e57127. https://doi.org/10.1371/journal.pone.0057127

Hazra, K. K., Nath, C. P., Singh, U., Praharaj, C. S., Kumar, N., Singh, S. S., & Singh, N. P. (2019). Diversification of maize-wheat cropping system with legumes and integrated nutrient management increases soil aggregation and carbon sequestration. *Geoderma*, *353*, 308-319. https://doi.org/10.1016/j.geoderma.2019.06.039

Ho, A., Di Lonardo, D. P., & Bodelier, P. L. (2017). Revisiting life strategy concepts in environmental microbial ecology. *FEMS microbiology ecology*, *93*(3). https://doi.org/10.1093/femsec/fix006

Hollister, E. B., Hu, P., Wang, A. S., Hons, F. M., & Gentry, T. J. (2013). Differential impacts of brassicaceous and nonbrassicaceous oilseed meals on soil bacterial and fungal communities. *FEMS microbiology ecology*, *83*(3), 632-641. https://doi.org/10.1111/1574-6941.12020

Hooper, D. U., & Dukes, J. S. (2004). Overyielding among plant functional groups in a long-term experiment. *Ecology Letters*, *7*(2), 95-105. https://doi.org/10.1046/j.1461-0248.2003.00555.x

Hooper, D. U., Chapin III, F. S., Ewel, J. J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setala, H., Symstad, A. J., Vandermeer, J., & Wardle, D. A. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological monographs*, *75*(1), 3-35. https://doi.org/10.1890/04-0922

Jabran, K., Mahajan, G., Sardana, V., & Chauhan, B. S. (2015). Allelopathy for weed control in agricultural systems. *Crop protection*, *72*, 57-65. https://doi.org/10.1016/j.cropro.2015.03.004

Jangid, K., Williams, M. A., Franzluebbers, A. J., Sanderlin, J. S., Reeves, J. H., Jenkins, M. B., Endale, D. M., Coleman, D. C., & Whitman, W. B. (2008). Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. *Soil Biology and Biochemistry*, *40*(11), 2843-2853. https://doi.org/10.1016/j.soilbio.2008.07.030

Jiao, X., Takishita, Y., Zhou, G., & Smith, D. L. (2021). Plant associated rhizobacteria for biocontrol and plant growth enhancement. *Frontiers in plant science*, *12*, 634796. https://doi.org/10.3389/fpls.2021.634796.

Kaye, J., Finney, D., White, C., Bradley, B., Schipanski, M., Alonso-Ayuso, M., Hunter, M., Burgess, M., & Mejia, C. (2019). Managing nitrogen through cover crop species selection in the US mid-Atlantic. *PLoS one*, *14*(4), e0215448. https://doi.org/10.1371/journal.pone.0215448

Kearney, S. P., Fonte, S. J., García, E., Siles, P., Chan, K. M. A., & Smukler, S. M. (2019). Evaluating ecosystem service trade-offs and synergies from slash-and-mulch agroforestry systems in El Salvador. *Ecological indicators*, *105*, 264-278. https://doi.org/10.1016/j.ecolind.2017.08.032.

Kim, N., Zabaloy, M. C., Guan, K., & Villamil, M. B. (2020). Do cover crops benefit soil microbiome? A meta-analysis of current research. *Soil Biology and Biochemistry*, *142*, 107701. https://doi.org/10.1016/j.soilbio.2019.107701

Kramer, S., Marhan, S., Ruess, L., Armbruster, W., Butenschoen, O., Haslwimmer, H., Kuzyakov, Y., Pausch, J., Scheunemann, N., Schoene, J., Schmalwasser, A., Totsche, K. U., Walker, F., Scheu, S., & Kandeler, E. (2012). Carbon flow into microbial and fungal biomass as a basis for the belowground food web of agroecosystems. *Pedobiologia*, *55*(2), 111-119. https://doi.org/10.1016/j.pedobi.2011.12.001

Lehman, R. M., Acosta-Martinez, V., Buyer, J. S., Cambardella, C. A., Collins, H. P., Ducey, T. F., Halvorson, J. J., Jin, V. L., Johnson, J. M. F., Kremer, R. J., Lundgren, J.G., Manter, D.K., Maul, J.E., Smith, J.L., & Stott, D. E. (2015a). Soil biology for resilient, healthy soil. *Journal of Soil and Water Conservation*, *70*(1), 12A-18A. https://doi.org/10.2489/jswc.70.1.12A

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. (2015b). Understanding and enhancing soil biological health: the solution for reversing soil degradation. *Sustainability*, *7*(1), 988-1027. https://doi.org/10.3390/su7010988

Lenth, R., Buerkner, P., Herve, M., Love, J., Miguez, F., Riebl, H., & Singmann, H. (2022). Emmeans: Estimated marginal means, aka least-squares means. R Package Version, 1.7.4-1.

Li, F., Sørensen, P., Li, X., & Olesen, J. E. (2020). Carbon and nitrogen mineralization differ between incorporated shoots and roots of legume versus non-legume based cover crops. *Plant and Soil*, *446*(1), 243-257. https://doi.org/10.1007/s11104-019-04358-6

Lin, H., & Peddada, S. D. (2020). Analysis of compositions of microbiomes with bias correction. *Nature communications*, *11*(1), 1-11. https://doi.org/10.1038/s41467-020-17041-7

Mackie, K. A., Schmidt, H. P., Müller, T., & Kandeler, E. (2014). Cover crops influence soil microorganisms and phytoextraction of copper from a moderately contaminated vineyard. *Science of the total environment*, *500*, 34-43. http://doi.org/10.1016/j.scitotenv.2014.08.091

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal*, *17*(1), 10-12. https://doi.org/10.14806/ej.17.1.200.

Martínez-García, L. B., Korthals, G., Brussaard, L., Jørgensen, H. B., & De Deyn, G. B. (2018). Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties. *Agriculture, Ecosystems & Environment*, *263*, 7-17. https://doi.org/10.1016/j.agee.2018.04.018

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS one*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217.

Meza, K., Vanek, S. J., Sueldo, Y., Olivera, E., Ccanto, R., Scurrah, M., & Fonte, S. J. (2022). Grass–legume mixtures show potential to increase above- and belowground biomass production for Andean forage-based fallows. *Agronomy*, *12*(1), 142. https://doi.org/10.3390/agronomy12010142

Millennium Ecosystem Assessment. (2005). *Ecosystems and Human Well-Being: Synthesis.* Island Press, Washington, DC.

Muhammad, I., Wang, J., Sainju, U. M., Zhang, S., Zhao, F., & Khan, A. (2021). Cover cropping enhances soil microbial biomass and affects microbial community structure: A meta-analysis. *Geoderma*, *381*, 114696. https://doi.org/10.1016/j.geoderma.2020.114696

Nelson, W. C., & Stegen, J. C. (2015). The reduced genomes of Parcubacteria (OD1) contain signatures of a symbiotic lifestyle. *Frontiers in Microbiology*, *6*, 713. https://doi.org/10.3389/fmicb.2015.00713

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., & Lüscher, A. (2011). Grass–legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agriculture, Ecosystems & Environment*, *140*(1-2), 155-163. https://doi.org/10.1016/j.agee.2010.11.022

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L. & Solymos, P. (2017). Vegan: community ecology package. R package version 2.4-3.

Paulson, J. N., Pop, M., & Bravo, H. C. (2013). metagenomeSeq: Statistical analysis for sparse high-throughput sequencing. *Bioconductor package*, *1*(0), 191.

Penn State Extension. (2020). *Cover Crop Mixtures: Calculating Seeding Rates.* https://extension.psu.edu/cover-crop-mixtures-calculating-seeding-rates

Pinheiro J, Bates D, R Core Team (2022). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-157.

Prescott, C. E., Frouz, J., Grayston, S. J., Quideau, S. A., & Straker, J. (2019). Rehabilitating forest soils after disturbance. In *Developments in Soil Science* (pp. 309-343). Elsevier. https://doi.org/10.1016/B978-0-444-63998-1.00013-6

Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., & Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research*, *35*(21), 7188-7196. https://doi.org/10.1093/nar/gkm864.

Qiao, Y. J., Li, Z. H., Wang, X., Zhu, B., Hu, Y. G., & Zeng, Z. H. (2012). Effect of legume-cereal mixtures on the diversity of bacterial communities in the rhizosphere. *Plant, Soil and Environment*, *58*(4), 174-180.

Rühlemann, L., & Schmidtke, K. (2015). Evaluation of monocropped and intercropped grain legumes for cover cropping in no-tillage and reduced tillage organic agriculture. *European Journal of Agronomy*, *65*, 83-94. https://doi.org/10.1016/j.eja.2015.01.006

Saleem, M., Pervaiz, Z. H., Contreras, J., Lindenberger, J. H., Hupp, B. M., Chen, D., Zhang, Q., Wang, C., Iqbal, J., & Twigg, P. (2020). Cover crop diversity improves multiple soil properties via altering root architectural traits. *Rhizosphere*, *16*, 100248. https://doi.org/10.1016/j.rhisph.2020.100248

Schipanski, M.E., Barbercheck, M., Douglas, M.R., Finney, D.M., Haider, K., Kaye, J.P., Kemanian, A.R., Mortensen, D.A., Ryan, M.R., Tooker, J., White, C. 2014. A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agricultural Systems, 125*, 12-22. https://doi.org/10.1016/j.agsy.2013.11.004.

Schlatter, D. C., Bakker, M. G., Bradeen, J. M., & Kinkel, L. L. (2015). Plant community richness and microbial interactions structure bacterial communities in soil. *Ecology*, *96*(1), 134-142. https://doi.org/10.1890/13-1648.1

Schmidt, R., Gravuer, K., Bossange, A. V., Mitchell, J., & Scow, K. (2018). Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. *PLoS one*, *13*(2), e0192953. https://doi.org/10.1371/journal.pone.0192953

Schnecker, J., Wild, B., Takriti, M., Alves, R. J. E., Gentsch, N., Gittel, A., Hofer, A., Klaus, K., Knoltsch, A., Lashchinskiy, N. & Richter, A. (2015). Microbial community composition shapes enzyme patterns in topsoil and subsoil horizons along a latitudinal transect in Western Siberia. *Soil Biology and Biochemistry*, *83*, 106-115. https://doi.org/10.1016/j.soilbio.2015.01.016

Seitz, V. A., McGivern, B. B., Daly, R. A., Chaparro, J. M., Borton, M. A., Sheflin, A. M., ... & Prenni, J. E. (2022). Variation in root exudate composition influences soil microbiome membership and function. *Applied and Environmental Microbiology*, e00226-22. https://doi.org/10.1128/aem.00226-22

Singh, G., Bhattacharyya, R., Das, T. K., Sharma, A. R., Ghosh, A., Das, S., & Jha, P. (2018). Crop rotation and residue management effects on soil enzyme activities, glomalin and aggregate stability under zero tillage in the Indo-Gangetic Plains. *Soil and Tillage Research*, *184*, 291-300. https://doi.org/10.1016/j.still.2018.08.006

Singh R. K., Singh, P, Li, H. B., Song, Q. Q., Guo, D. J., Solanki, M. K., Verma, K. K., Malviya, M.K., Song X. P., Lakshmanan, P., Yang, L. T., & Li, Y. R. (2020). Diversity of nitrogen-fixing rhizobacteria associated with sugarcane: a comprehensive study of plant-microbe interactions for growth enhancement in Saccharum spp. *BMC Plant Biology*, *20*(1), 1-21. https://doi.org/10.1186/s12870-020-02400-9.

Sinsabaugh, R. L., Lauber, C. L., Weintraub, M. N., Ahmed, B., Allison, S. D., Crenshaw, C., ... & Zeglin, L. H. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecology letters*, *11*(11), 1252-1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Soil Survey Staff. (2020). *Web Soil Survey.* U.S. Department of Agriculture, National Resources Conservation Service. http://www.websoilsurvey.nrcs.usda.gov

Tamburini, G., Bommarco, R., Wanger, T. C., Kremen, C., Van Der Heijden, M. G., Liebman, M., & Hallin, S. (2020). Agricultural diversification promotes multiple ecosystem services without compromising yield. *Science advances*, *6*(45). https://doi.org/10.1126/sciadv.aba1715

Trivedi, P., Anderson, I. C., & Singh, B. K. (2013). Microbial modulators of soil carbon storage: integrating genomic and metabolic knowledge for global prediction. *Trends in Microbiology*, *21*(12), 641–651. https://doi.org/10.1016/j.tim.2013.09.005

Trivedi, P., Delgado-baquerizo, M., Jeffries, T. C., Trivedi, C., Anderson, I. C., Lai, K., Mcnee, M., Flower, K., Singh, B. P., Minkey, D., & Singh, B. K. (2017). Soil aggregation and associated microbial communities modify the impact of agricultural management on carbon content. *Environmental microbiology*, *19*(8), 3070-3086. https://doi.org/10.1111/1462-2920.13779

Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., & Singh, B. K. (2020). Plant–microbiome interactions: from community assembly to plant health. *Nature reviews microbiology*, *18*(11), 607-621. https://doi.org/10.1038/s41579-020-0412-1

Udom, B. E., & Omovbude, S. (2019). Soil physical properties and carbon/nitrogen relationships in stable aggregates under legume and grass fallow. *Acta Ecologica Sinica*, *39*(1), 56-62. https://doi.org/10.1016/j.chnaes.2018.05.008

Velásquez, E., Fonte, S. J., Barot, S., Grimaldi, M., Desjardins, T., & Lavelle, P. (2012). Soil macrofauna-mediated impacts of plant species composition on soil functioning in Amazonian pastures. *Applied Soil Ecology*, *56*, 43-50. https://doi.org/10.1016/j.apsoil.2012.01.008

Van Bavel, C. H. M. (1950). Mean weight-diameter of soil aggregates as a statistical index of aggregation. *Proceedings. Soil Science Society of America, 1949*, *14*, 20-23. https://doi.org/10.2136/ sssaj1950.036159950014000C0005x.

Wang, Z., Wang, Z., Li, T., Wang, C., Dang, N., Wang, R., Jiang, Y., Wang, H. & Li, H. (2021). N and P fertilization enhanced carbon decomposition function by shifting microbes towards an r-selected community in meadow grassland soils. *Ecological Indicators*, *132*, 108306. https://doi.org/10.1016/j.ecolind.2021.108306

Weil, R. R., Islam, K. R., Stine, M. A., Gruver, J. B., & Samson-Liebig, S. E. (2003). Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. *American Journal of Alternative Agriculture*, 3-17. https://doi.org/10.1079/AJAA200228

Wick, A., Berti, M., Lawley, Y., & Liebig, M. (2017). Integration of annual and perennial cover crops for improving soil health. In *Soil health and intensification of agroecosystems* (pp. 127-150). Academic Press.

Xiong, C., He, J.Z., Singh, B.K., Zhu, Y.G., Wang, J.T., Li, P.P., Zhang, Q.B., Han, L.L., Shen, J.P., Ge, A.H., Wu, C.F., & Zhang, L.M. (2021a). Rare taxa maintain the stability of crop mycobiomes and ecosystem functions. *Environmental Microbiology*, *23*(4), 1907-1924. https://doi.org/10.1111/1462-2920.15262.

Xiong, C., Zhu, Y.G., Wang, J.T., Singh, B., Han, L.L., Shen, J.P., Li, P.P., Wang, G.B., Wu, C.F., Ge, A.H., Zhang, L.M., & He, J.Z. (2021b). Host selection shapes crop microbiome assembly and network complexity. *New Phytologist*, *229*(2), 1091-1104. https://doi.org/10.1111/nph.16890.

Zheng, Q., Hu, Y., Zhang, S., Noll, L., Böckle, T., Dietrich, M., Herbold, C.W., Eichorst, S.A., Woebken, D., Richter, A. & Wanek, W. (2019). Soil multifunctionality is affected by the soil environment and by microbial community composition and diversity. *Soil Biology and Biochemistry*, *136*, 107521. https://doi.org/10.1016/j.soilbio.2019.107521

Zuo, S., Li, X., Ma, Y., & Yang, S. (2014). Soil microbes are linked to the allelopathic potential of different wheat genotypes. *Plant and soil*, *378*(1), 49-58. https://doi.org/10.1007/s11104-013-2020-6

APPENDIX: SUPPLEMENTARY TABLES AND FIGURES

Table S2.1. Regional distribution of area with continued and new cover crop (CC) adoption if CC adoption rates were increased to a target 80% per region. Total area included in the study is also presented, as well as adoption rates under baseline and high CC adoption scenarios. Means of the six management imputations are present, with standard deviations indicated in parentheses.

Region	Total Area Mha		New CC Adoption Area Mha		Continued CC Adoption Area Mha		Baseline Adoption Rates %	High Adoption Rates %
NW Coastal	0.02	(0.00)	0.01	(0.00)	0.00	(0.00)	2.7	73.2
CA Coastal	0.4	(0.00)	0.29	(0.01)	0.02	(0.00)	4.8	81.2
NW Non-Coastal	0.9	(0.00)	0.73	(0.00)	0.01	(0.00)	1.6	79.0
SW Non-Coastal	0.3	(0.00)	0.22	(0.00)	0.00	(0.00)	1.5	72.0
N Great Plains	15.2	(0.00)	11.0	(0.03)	0.22	(0.01)	1.5	73.9
S Great Plains	14.5	(0.00)	12.5	(0.02)	0.47	(0.02)	3.3	89.4
N Central	44.9	(0.00)	36.1	(0.18)	2.12	(0.01)	4.7	85.1
S Central	1.5	(0.00)	1.16	(0.01)	0.03	(0.00)	2.0	80.3
Lower MS	7.3	(0.00)	5.60	(0.07)	0.31	(0.01)	4.2	80.6
Northeast	2.2	(0.00)	1.52	(0.00)	0.22	(0.00)	10.1	78.3
E Central	3.2	(0.00)	2.43	(0.01)	0.25	(0.01)	7.8	82.6
Southeast	3.6	(0.00)	2.63	(0.14)	0.43	(0.01)	12.1	86.0
National	**94.1**	**(0.00)**	**74**	**(0.03)**	**4.1**	**(0.19)**	**4.4**	**83.2**

Table S2.2. Cover crop (CC) type selection rules, most prevalent cropping system, and mean number of CC harvests by type for each region throughout the 20-year study period.

Region	Rules for Selection of Cover Crop Type	Common Cropping System	Total CC Harvests	Total Legume CC Harvests	Total Grass CC Harvests	Total Radish CC Harvests
NW Coastal	If harvest before mid-September and next crop is not a legume plant clover, else cereal rye	Corn	9.5	7.7	2.42	0.00
CA Coastal	If harvest before mid-October and next crop is not a legume plant clover, else cereal rye	Corn, Rice	12.3	12.5	0.10	0.00
NW Non-Coastal	If harvest before mid-September and next crop is not a legume plant clover, else cereal rye	Wheat, Potato	11.1	10.2	1.20	0.00
SW Non-Coastal	If harvest before late August and next crop is not a legume plant clover, else cereal rye	Wheat, Cotton, Alfalfa	10.6	4.3	6.78	0.00
N Great Plains	If harvest before mid-August and next crop is not a legume plant clover else radish, else cereal rye	Corn, Soy, Wheat, Sunflower	12.9	4.4	7.78	0.69
S Great Plains	If harvest before late August and next crop is not a legume plant clover else radish, else cereal rye	Corn, Soy, Wheat, Cotton, Sorghum	10.4	3.2	6.61	0.62
N Central	If harvest before late August and next crop is not a legume plant clover else radish, else cereal rye	Corn, Soy	13.9	0.8	12.11	1.05
S Central	If harvest before mid-September and next crop is not a legume plant clover else radish, else cereal rye	Corn, Soy, Wheat	10.6	5.5	2.81	2.27
Lower MS	If harvest before mid-October and next crop is not a legume plant clover, else cereal rye	Corn, Soy, Wheat, Cotton	12.9	5.7	7.34	0.00
Northeast	If harvest before mid-August and next crop is not a legume plant clover, else cereal rye	Corn, Soy, Wheat	12.9	1.0	12.34	0.00
E Central	If harvest before late August and next crop is not a legume plant clover, else cereal rye	Corn, Soy, Wheat	13.6	2.7	11.23	0.00
Southeast	If harvest before mid-October and next crop is not a legume plant clover, else cereal rye	Corn, Soy, Wheat, Cotton	12.4	6.1	6.36	0.00

Table S2.3. Mean annual C sequestration, N_2O emissions, and net greenhouse gas (GHG) flux from 2020 to 2039 for the baseline cover crop (CC) adoption scenario and the change due to high CC adoption. The mean of 1000 Monte-Carlo iterations is presented with the standard deviation in parentheses.

	Net GHG Flux				C sequestration				N_2O emissions			
	Mt CO_2e year^{-1}				Mt CO_2e year^{-1}				Mt CO_2e year^{-1}			
Region	Baseline		Change with High Adoption		Baseline		Change with High Adoption		Baseline		Change with High Adoption	
NW Coastal	0.02	(0.01)	-0.01	(0.02)	0.00	(0.01)	-0.01	(0.02)	0.02	(0.00)	0.00	(0.01)
CA Coastal	0.35	(0.08)	-0.19	(0.13)	-0.02	(0.05)	-0.25	(0.10)	0.37	(0.05)	0.06	(0.08)
NW Non-Coastal	1.12	(0.24)	-0.23	(0.34)	-0.11	(0.08)	-0.23	(0.15)	1.23	(0.23)	0.00	(0.30)
SW Non-Coastal	0.43	(0.09)	-0.08	(0.12)	0.02	(0.07)	-0.09	(0.09)	0.41	(0.05)	0.00	(0.07)
N Great Plains	13.60	(2.77)	-4.08	(3.94)	-1.72	(0.61)	-3.78	(1.60)	15.31	(2.71)	-0.30	(3.65)
S Great Plains	12.73	(2.33)	-5.69	(3.75)	-2.55	(0.71)	-5.12	(2.08)	15.27	(2.27)	-0.57	(3.06)
N Central	55.72	(10.9)	-17.98	(15.87)	-4.56	(1.37)	-15.77	(5.71)	60.29	(10.8)	-2.20	(14.59)
S Central	1.93	(0.26)	-0.79	(0.44)	-0.06	(0.11)	-0.88	(0.28)	1.99	(0.24)	0.09	(0.34)
Lower MS	7.63	(1.16)	-5.67	(2.30)	-0.92	(0.32)	-5.76	(1.75)	8.55	(1.12)	0.09	(1.55)
Northeast	2.73	(0.40)	-0.78	(0.55)	0.40	(0.15)	-0.73	(0.21)	2.33	(0.37)	-0.05	(0.50)
E Central	3.17	(0.38)	-1.71	(0.66)	0.06	(0.14)	-1.66	(0.44)	3.12	(0.36)	-0.05	(0.50)
Southeast	2.01	(0.35)	-1.75	(0.75)	-0.48	(0.15)	-1.84	(0.61)	2.49	(0.32)	0.09	(0.45)
National	**101.4**	**(16.9)**	**-38.96**	**(24.27)**	**-9.94**	**(1.96)**	**-36.11**	**(7.88)**	**111.4**	**(16.7)**	**-2.85**	**(22.78)**

Table S2.4. Cover crop (CC) type selection rules, most prevalent cropping system, and number of CC harvests by type for each region throughout the 20-year study period. Mean of all study locations is presented with the standard deviation in parentheses.

Region	Sand Content		Annual CC Biomass C		Potential Evapotranspiration		Mean Annual Precipitation		Tillage		Annual N Fertilizer	
	%		kg C ha^{-1} yr^{-1}		mm		mm		(NT = 0; RT = 1; CT = 2)		kg N ha^{-1} yr^{-1}	
NW Coastal	23.5	(17.2)	750	(372)	692	(64)	1176	(189)	1.29	(0.35)	117.6	(18.9)
CA Coastal	26.4	(20.9)	1363	(719)	1072	(37)	400	(121)	1.39	(0.37)	40.0	(12.1)
NW Non-Coastal	33.0	(23.9)	546	(316)	798	(58)	311	(109)	1.15	(0.39)	31.1	(10.9)
SW Non-Coastal	35.0	(21.1)	574	(390)	1167	(172)	245	(80)	1.76	(0.36)	24.5	(8.0)
N Great Plains	33.5	(19.2)	557	(304)	683	(58)	554	(79)	1.06	(0.45)	55.4	(7.9)
S Great Plains	31.5	(24.8)	717	(300)	964	(113)	621	(145)	0.99	(0.48)	62.1	(14.5)
N Central	20.3	(19.5)	647	(262)	748	(58)	980	(159)	1.03	(0.37)	98.0	(15.9)
S Central	20.4	(16.8)	1156	(426)	993	(95)	1151	(215)	0.77	(0.51)	115.1	(21.5)
Lower MS	16.3	(17.6)	1695	(523)	944	(61)	1370	(136)	0.81	(0.48)	137.0	(13.6)
Northeast	30.8	(15.0)	870	(478)	745	(55)	1150	(112)	1.21	(0.42)	115.0	(11.2)
E Central	22.2	(19.9)	1111	(392)	864	(65)	1299	(134)	0.91	(0.39)	129.9	(13.4)
Southeast	64.6	(23.9)	1294	(402)	962	(96)	1306	(119)	0.91	(0.43)	130.6	(11.9)

Table S4.1. Planting, cover crop (CC) termination and winter wheat (WW) harvest dates for two adjacent dryland cover crop field trials (T1 and T2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado.

		Trial	
		T1	**T2**
Cycle 1	CC Planting Date	Sept. 28, 2015	Fall-planted: Aug. 11, 2016 Spring-planted Apr. 13, 2017
	CC Termination Date	June 10, 2016	June 20, 2017
	WW Planting Date	Sept. 19, 2016	Sept. 21, 2017
	WW Harvest Date	June 20, 2017	July 25, 2018
Cycle 2	CC Planting Date	Aug. 30, 2017	Fall-planted: Sept. 5, 2018 Spring-planted: May 7, 2019
	CC Termination Date	June 8, 2018	Fall-planted: June 19, 2019 Spring-planted: July 10, 2019
	WW Planting Date	Sept. 28, 2018	Oct. 23, 2019
	WW Harvest Date	Aug 7, 2019	July 27, 2020
Cycle 3	CC Planting Date	Sept. 13, 2019	Fall-planted: Sept. 16, 2020 Spring-planted: April 29, 2021
	CC Termination Date	June 18, 2020	Fall-planted: June 12, 2021 Spring-planted: July 13, 2021
	WW Planting Date	Sept. 18, 2020	Sept. 7, 2021
	WW Harvest Date	July 27, 2021	NA [1]

[1] NA, not applicable. Project ended before third winter wheat harvest.

Table S4.2. Revenue, expenses and net returns following three cover crop (CC) – winter wheat cycles (C1, C2 & C3) at two adjacent dryland cover crop field trials (T1 and T2) at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Treatments consist of winter wheat rotated with either a fall-planted CC mix, a spring-planted CC mix, or bare fallow and managed under no-till (NT) or conventional tillage (CT). Net returns were evaluated with and without the sale of 50% of CC biomass as forage. All values are displayed in USD ha^{-1} $cycle^{-1}$.

Trial	Tillage	CC	Cycle	Wheat and CC Expenses		Wheat Yield and Prices			Forage Expenses	Forage Yield and Prices			Net Returns	
				Operating	Ownership	Yield	Price	Revenue	Operating and Ownership	Yield	Price	Revenue	Without Forage Revenue	With Forage Revenue
				USD ha^{-1}	USD ha^{-1}	bu ha^{-1}	USD bu^{-1}	USD ha^{-1}	USD ha^{-1}	t ha^{-1}	USD t^{-1}	USD ha^{-1}	USD ha^{-1}	USD ha^{-1}
T1	NT	Fallow	C1	131.46	81.79	151.7	3.00	423.23	NA	NA	NA	NA	209.99	209.99
T1	NT	Fallow	C2	111.04	66.99	46.1	3.71	169.43	NA	NA	NA	NA	-8.59	-8.59
T1	NT	Fallow	C3	105.74	64.87	44.2	5.63	250.99	NA	NA	NA	NA	80.39	80.39
T1	NT	Fall	C1	224.45	94.52	110.7	3.00	308.81	100.89	3.25	150.00	488.08	-10.16	377.03
T1	NT	Fall	C2	178.46	75.90	51.1	3.71	187.60	73.62	0.92	150.10	146.99	-66.76	6.61
T1	NT	Fall	C3	169.68	73.67	8.8	5.63	50.03	71.57	0.72	165.88	130.07	-193.33	-134.83
T2	NT	Fallow	C1	104.17	78.73	58.8	3.00	164.05	NA	NA	NA	NA	-18.85	-18.85
T2	NT	Fallow	C2	95.66	64.80	44.0	3.71	161.77	NA	NA	NA	NA	1.31	1.31
T2	NT	Fall	C1	156.66	86.09	16.0	3.00	44.67	100.89	2.52	150.00	377.37	-198.09	78.39
T2	NT	Fall	C2	156.51	73.13	19.6	3.71	72.20	73.62	2.86	150.10	457.59	-157.44	226.52
T2	NT	Spring	C1	180.44	88.99	45.3	3.00	126.37	100.89	0.41	150.00	61.97	-143.05	-181.97
T2	NT	Spring	C2	191.73	77.70	30.8	3.71	113.24	73.62	1.28	150.10	204.13	-156.19	-25.69
T2	CT	Fallow	C1	74.84	79.79	35.1	3.00	97.88	NA	NA	NA	NA	-56.75	-56.75
T2	CT	Fallow	C2	67.07	64.77	41.9	3.71	154.11	NA	NA	NA	NA	22.27	22.27
T2	CT	Fall	C1	147.85	89.77	5.3	3.00	14.65	100.89	2.48	150.00	371.92	-222.96	48.07
T2	CT	Fall	C2	125.58	72.78	19.1	3.71	70.11	73.62	2.94	150.10	470.98	-128.25	269.11
T2	CT	Spring	C1	129.74	87.14	29.6	3.00	82.72	100.89	0.55	150.00	82.20	-134.16	-152.85
T2	CT	Spring	C2	143.49	75.00	34.1	3.71	125.16	73.62	1.25	150.10	199.33	-93.34	32.38

Table S4.3. Mean values of ecosystem service proxies by cover crop (CC) treatment mix from three CC cycles of a field trial (T1) located at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Treatments consist of winter wheat rotated with one of three fall-planted CC mix or a bare fallow control. Values with different lowercase letters (by column) indicate differences to an alpha level of 0.05, as determined by Tukey-adjusted multiple comparisons.

	2016-2017 Cycle				2018-2019 Cycle				2020-2021 Cycle				End of Trial	
CC Mix	CC Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield	CC Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield	CC Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield	Total C	Aggregation (MWD [1])
	kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}	kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}	kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}	%	um
Mix 1	4560 ^{a}	9.68 ^{a}	157 ^{a}	3.00 ^{a}	1410 ^{a}	13.6 ^{ab}	129 ^{a}	1.57 ^{a}	1150 ^{a}	3.14 ^{a}	138 ^{a}	0.14 ^{a}	0.80 ^{a}	262 ^{a}
Mix 2	4860 ^{a}	10.2 ^{a}	151 ^{a}	3.01 ^{a}	1570 ^{a}	11.0 ^{a}	120 ^{a}	1.47 ^{ab}	1080 ^{a}	4.13 ^{a}	137 ^{a}	0.26 ^{a}	0.87 ^{a}	214 ^{ab}
Mix 3	5650 ^{a}	NE [1]	163 ^{a}	2.82 ^{a}	1550 ^{a}	12.7 ^{a}	119 ^{a}	1.51 ^{ab}	1400 ^{a}	3.98 ^{a}	127 ^{a}	0.27 ^{a}	0.78 ^{a}	222 ^{ab}
Fallow	NE [2]	17.1 ^{b}	224 ^{b}	4.03 ^{b}	NE	19.4 ^{b}	131 ^{a}	1.35 ^{b}	NE	6.04 ^{a}	176 ^{b}	1.14 ^{b}	0.78 ^{a}	185 ^{b}
ANOVA [3]														
	0.60	0.005	0.010	<0.0001	0.60	0.013	0.38	0.028	0.32	0.22	0.004	<0.001	0.36	0.06

[1] MWD, mean weight diameter. [2] NE, not evaluated. [3] Differences analyzed using a multifactor analysis of variance (ANOVA) with CC mix included as a fixed effect and block included as a random effect. *p*-values presented.

Table S4.4. Mean values of ecosystem service proxies by tillage and cover crop (CC) treatment mix from two CC cycles of a field trial (T2) located at the Southwestern Colorado Research Center near Yellow Jacket, Colorado. Treatments include winter wheat rotated with one of five fall-planted cover crop mixtures (Mix 1-5), one of three spring-planted mixtures (Mix 6-8) or a fallow control and managed under no-till (NT) or conventional tillage (CT). Values with different lowercase letters (by column) indicate differences to an alpha level of 0.05, as determined by Tukey-adjusted multiple comparisons.

			2017-2018 Cycle				2019-2020 Cycle				End of Trial	
CC Planting Window	CC Mix	Tillage	CC Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield	CC Biomass	Soil Nitrate	Soil Water Storage	Wheat Yield	Total C	Aggregation (MWD[1])
			kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}	kg ha^{-1}	mg kg^{-1}	mm	Mg ha^{-1}	%	um
Fall	Mix 1	CT	3620^{ab}	3.28^{a}	149^{abc}	0.11^{ab}	5170^{ab}	NE	NE	0.56	NE	NE
		NT	4120^{a}	4.57^{ab}	147^{ab}	0.42^{abcd}	5050^{ab}	NE	NE	0.54	NE	NE
	Mix 2	CT	3570^{ab}	NE[2]	NE	0.08^{a}	4800^{abc}	1.01^{a}	121^{ab}	0.51	0.69^{a}	302^{a}
		NT	3940^{a}	NE	NE	0.34^{abcd}	5110^{ab}	0.86^{a}	108^{a}	0.59	0.60^{a}	293^{ab}
	Mix 3	CT	3660^{ab}	NE	NE	0.22^{abc}	4710^{abcd}	0.54^{a}	117^{a}	0.47	0.71^{a}	267^{ab}
		NT	3840^{a}	NE	NE	0.41^{abcd}	4600^{abcd}	1.33^{a}	113^{a}	0.45	0.71^{a}	255^{ab}
	Mix 4	CT	4490^{a}	NE	NE	0.14^{abc}	5240^{a}	NE	NE	0.50	NE	NE
		NT	3520^{ab}	NE	NE	0.59^{abcde}	4450^{abcd}	NE	NE	0.53	NE	NE
	Mix 5	CT	3760^{ab}	3.01^{a}	137^{a}	0.15^{abc}	4270^{abcd}	0.76^{a}	116^{a}	0.57	0.62^{a}	294^{ab}
		NT	3970^{a}	4.16^{ab}	151^{ab}	0.42^{abcd}	4300^{abcd}	1.07^{a}	113^{a}	0.59	0.71^{a}	280^{ab}
Spring	Mix 6	CT	855^{c}	5.39^{b}	177^{abcd}	0.77^{abcdef}	1950^{cd}	1.58^{ab}	132^{ab}	0.82	0.71^{a}	233^{ab}
		NT	742^{c}	5.37^{b}	173^{abcd}	1.00^{bcdef}	2110^{cd}	1.58^{a}	128^{ab}	0.84	0.69^{a}	236^{ab}
	Mix 7	CT	556^{c}	NE	NE	0.81^{bcdef}	1800^{d}	NE	NE	0.98	NE	NE
		NT	440^{c}	NE	NE	1.24^{ef}	2310^{bcd}	NE	NE	0.82	NE	NE
	Mix 8	CT	1120^{bc}	5.30^{b}	194^{bcd}	0.84^{cdef}	2400^{abcd}	NE	NE	0.99	NE	NE
		NT	728^{c}	5.48^{b}	185^{abcd}	1.33^{f}	1870^{d}	NE	NE	0.88	NE	NE
NA	Fallow	CT	NE	9.15^{c}	211^{d}	0.95^{def}	NE	1.25^{a}	153^{b}	1.17	0.62^{a}	240^{ab}
		NT	NE	9.61^{c}	209^{cd}	1.60^{f}	NE	3.43^{b}	156^{b}	1.26	0.70^{a}	202^{b}
						ANOVA [3]						
		CC Mix	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	0.13	0.002
		Tillage	1.00	0.129	0.880	0.102	0.83	0.15	0.52	0.94	0.80	0.36
		CC Mix x Tillage	0.86	0.377	0.740	0.738	0.95	0.02	0.79	0.50	0.02	0.86

[1] MWD, mean weight diameter. [2] NE, not evaluated. [3] Differences analyzed using a multifactor analysis of variance (ANOVA) with CC mix and tillage included as fixed effects and block and tillage split-plots included as random effects. *p*-values presented.

Table S5.1. Cover crop (CC) seeding rates for CC treatments with different functional compositions in a greenhouse study. Treatments included factorial combinations of CC functional types [Poaceae (P), Fabaceae (F), Brassicaceae (B), and Asteraceae (A)] with functional diversity (FD) varying between one and four types. Seeding rates are displayed in seeds pot^{-1} with equivalent kg ha^{-1} rate in parentheses.

	Seeding rate [a]							
Treatment	Annual Ryegrass	Triticale	Common Vetch	Winter Pea	Rapeseed	Forage Radish	Sunflower	Safflower
P	10 (19)	5 (100)						
F			4 (130)	2 (148)				
B					6 (16)	3 (24)		
A							2 (87)	2 (40)
PF	5 (9.4)	3 (60)	2 (65)	2 (148)				
PB	5 (9.4)	3 (60)			3 (7.9)	2 (16)		
PA	5 (9.4)	3 (60)					1 (44)	1 (20)
FB			2 (65)	2 (148)	3 (7.9)	2 (16)		
FA			2 (65)	2 (148)			1 (44)	1 (20)
BA					3 (7.9)	2 (16)	1 (44)	1 (20)
PFB	4 (7.5)	2 (40)	2 (65)	1 (74)	2 (5.3)	2 (16)		
PFA	4 (7.5)	2 (40)	2 (65)	1 (74)			1 (44)	1 (20)
PBA	4 (7.5)	2 (40)			2 (5.3)	2 (16)	1 (44)	1 (20)
FBA			2 (65)	1 (74)	2 (5.3)	2 (16)	1 (44)	1 (20)
PFBA	3 (5.6)	1 (20)	1 (32)	1 (74)	1 (2.6)	1 (8.0)	1 (44)	1 (20)

[a] Seeding rates were determined based on recommended monoculture seeding rates and seeding guidelines for CC mixtures (Penn State Extension, 2020).

Table S5.2. Means of ES proxies for cover crop (CC) treatments of varying functional composition in a greenhouse study. Treatments included factorial combinations of CC functional types [Poaceae (P), Fabaceae (F), Brassicaceae (B), and Asteraceae (A)] with functional diversity (FD) varying between one and four types. Values represent the mean of each treatment.

FC	Soil Structure	Soil Fertility	C-Cycling		N-Cycling			P-Cycling	Microbial Community			
	MWD [1]	POXC [2]	CB [3]	BG [4]	NAG [5]	LAP [6]	TAP [7]	PHOS [8]	Alpha Diversity	Richness	Evenness	Oligo:Copio [9]
	um	mg C kg^{-1}	nmol h^{-1} g^{-1}	nmol h^{-1} g^{-1}	nmol h^{-1} g^{-1}	nmol h^{-1} g^{-1}	nmol h^{-1} g^{-1}	nmol h^{-1} g^{-1}	Shannon	OTU count	Pielou's	Ratio
P	753	175	4.29	38.5	7.9	20.8	13.0	64.5	7.31	2945	0.916	0.756
F	769	147	6.04	48.9	10.9	26.2	15.5	76.4	7.09	2694	0.898	0.688
B	752	186	6.68	50.6	10.1	21.1	15.0	67.1	7.37	3093	0.917	0.720
A	745	175	5.18	43.8	8.8	20.1	12.2	65.4	7.29	2952	0.913	0.714
PF	761	180	5.64	48.9	10.1	25.8	16.6	68.6	7.29	2950	0.912	0.697
PB	756	158	3.90	42.5	7.4	22.3	15.9	62.9	7.40	3057	0.922	0.726
PA	758	164	5.30	42.7	11.5	23.1	12.9	66.6	7.32	2988	0.915	0.705
FA	759	147	5.62	46.6	9.7	22.5	14.1	66.7	7.14	2802	0.900	0.692
FB	768	175	6.62	50.5	14.0	28.4	17.9	75.1	7.22	2880	0.906	0.690
BA	749	169	6.83	48.5	11.2	23.6	15.1	67.8	7.28	2996	0.909	0.718
PFA	760	169	6.03	45.3	10.7	26.1	18.0	69.1	7.28	2931	0.912	0.701
PFB	756	150	7.55	51.6	11.5	23.5	15.6	71.5	7.31	2965	0.914	0.710
PBA	753	160	6.59	47.9	10.1	24.1	16.8	62.3	7.37	3028	0.920	0.724
FBA	768	170	7.29	52.8	12.1	23.2	13.3	72.1	7.24	2887	0.908	0.699
PFBA	762	167	6.01	48.1	10.6	25.8	16.6	71.1	7.28	2968	0.910	0.695

[1] MWD, mean weight diameter. [2] POXC, permanganate-oxidizable C. [3] CB, β-cellobiosidase. [4] BG, β-glucosidase. [5] NAG, β-N-acetylglucosaminidase. [6] LAP, leucine aminopeptidase. [7] TAP, tyrosine aminopeptidase. [8] PHOS, phosphatase. [9] Oligo:Copio, ratio of oligotrophic to copiotrophic microbes.