Cover crop effects on the carbon cycle

By

Anna Marie Cates

A dissertation submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

(Agronomy)

At the

UNIVERSITY OF WISCONSIN-MADISON

2018

Date of final oral examination: 5/24/2018

The dissertation is approved by the members of the Final Oral Committee:

Randall D. Jackson, Professor, Agronomy

Claudio Gratton, Professor, Entomology

William D. Hickey, Professor, Soil Science

Christopher J. Kucharik, Associate Professor, Agronomy

Erika Marin-Spiotta, Associate Professor, Geography

Abstract

Cover crops, or the practice of adding an unharvested crop to an annual crop rotation, are increasing in popularity and widely recommended to help retain soil, water, and nutrients on agricultural land. However, the ability of cover crops to improve soil health and sequester soil C remains unknown. The difficulty of establishing productive cover crops in northern climates creates additional incentive to investigate the effects of cover crops on the C cycle in northern systems. Cover crops are thought likely to increase soil C because they add diversity in C inputs and increase the total growing season, but the magnitude and probability of this effect in different systems is critical for informing our understanding of cover crop efficacy and making recommendations to growers. In addition, cover crops may impact biogeochemical cycling and soil processes via changing the abiotic environment. Motivated by the need for detailed evidence of cover crop efficacy at multiple scales, I combined field-based evaluation of cover crop effects on the C cycle with an investigation into mechanisms of C storage under varying abiotic conditions.

In Chapter 1, my co-authors and I review the state of cover cropping in the North Central U.S. and suggest that expectations of cover crops be restrained to the proven benefits related to water, soil, and nutrient retention. We review establishment constraints and variability in production to argue that cover cropping in northern climates requires specific agronomic systems and "buy-in" from farmers and government agencies.

In Chapter 2, we evaluated the net ecosystem C balanced (NECB) of maize-cover crop systems. We used a biometric approach to evaluate whether rye or bluegrass cover crops with grain or silage maize increased the total C inputs of net primary productivity (NPP) or removal as harvested yield or heterotrophic respiration (R_h). We found that grain maize's NECB hovered near zero, suggesting that with or without cover crops the systems had marginal ability to increase soil C. Silage maize's NECB was

always negative, despite lower maize residue allowing for five-fold greater rye. Bluegrass grew equally well under maize or silage, but depressed silage yield. Cover crops did not affect annual R_h.

In Chapter 3, we examined whether cover crops affected other components of the C cycle. I carried out a litterbag experiment to evaluate whether cover crops changed the rate of maize residue decomposition or soil or litter microbial decomposer community. After 4 years of cover cropping I analyzed particulate organic matter (POM) and potentially mineralizable C (PMC) as indices of active C, which may be increased by cover crop root exudates and microbial stimulation. We found that cover crops did not alter soil or litter microbial composition according to shotgun metagenomics or maize residue decomposition rate, which is an important management consideration for northern farmers. We observed increases in PMC and POM-C with cover crops, which were correlated with total NPP, confirming that active C pools may be more sensitive to management and C input increases in particular.

In Chapter 4, we report findings of a laboratory incubation evaluating whether temperature and moisture effect on physical protection of decomposing plant litter C. Cover crops and other agronomic management may affect soil temperature and moisture yet we have little understanding of how these conditions shift not just the rate of C mineralization, but the fate of C not mineralized. I traced plant litter C into aggregate fractions over six months of incubation, and evaluated microbial community, biomass, enzyme activity and aggregate C compounds. We found that higher temperatures increased C mineralization, shifted bacterial composition, and decreased efficiency of conversion of litter C to soil C, but dry conditions increased soil aggregation. This novel result emphasizes that whilethe temperature regime may drive microbial C mineralization, the moisture regime may drive physical protection and long-term stability of soil C.

Acknowledgements

It has been a privilege to spend these years working on exciting research with supportive colleagues, and I'll always be grateful to Dr. Randy Jackson for providing the opportunity. Randy has a special talent for drawing creative and critical minds together, and I've grown tremendously through my time in the Jackson Lab. In addition, Randy has had my back in every way, and I'm grateful for his unwavering support as well as his big-picture perspective on every question. The Great Lakes Bioenergy Research Center and the Smith Family Fellowship fully funded my work, and the freedom I've had to explore any question that took my fancy is a great privilege.

My committee members have all contributed to my development and work in important ways. Erika Marin-Spiotta set me on a path of fascination with soil organic matter and has always been willing to talk about soil aggregates, C, microbes and minerals. Chris Kucharik has provided important perspective on methods and given me a wonderful opportunity to work with him the classroom. Bill Hickey's door and lab were always open to me, and I'm grateful for his generous support of my lab work and the example he provides of a professor who still gets to do bench science. Claudio Gratton opened my eyes wide to arthropods and multivariate statistics, and his cheerful presence in WEI is always uplifting.

In addition, several other scientists at UW-Madison have helped me along the way. Nick Keuhler provided statistical consulting. Francisco Arriaga lent me equipment. Gary Oates ran samples, coordinated humans, and helped keep the whole machine running smoothly. Thea Whitman engaged in my work from the moment she stepped on campus and contributed greatly to the experimental design and scientific framing of Chapter 3. Matt Ruark, my M.S. advisor, has a great mix of practical and scientific perspective and was always willing to talk about my adventures after I left his lab. Gregg Sanford's encouragement and enthusiasm for agronomy, science, and life in general was a great support to me.

My fellow Jackson Lab students David Duncan, Laura Smith, Greta Landis, and Adam von Haden all contributed words of advice and empathy over the years. Adam in particular helped me out of many a technical jam with Li-Cor as well as filling important gaps left in my work with his own, and David's critical perspective on the scientific endeavor has been important in my own development as a scientist.

I probably can't thank every pair of hands who touched the samples underpinning the data in this work, but to start with I'm grateful to Sarah Lee, who kept the projects running in the summer of 2016, Kelsey Johnson, who capably managed so much data collection that 2017 breezed by, Ollie Fisher, who washed 90% of the roots we collected over the years by himself in the basement of Moore Hall, and Megan Lipke, whose enthusiasm for the science behind the weighing, scraping and rolling gave me some needed boosts. Allison Dungan and Adam Mittermaier stepped above and beyond being my best friends to pull litterbags with me when I was desperate. James Tesmer, Alex Butz, Jimmy Sustachek, Mark Walsh and Casey Menick were reliable connections to equipment and field conditions, ensuring that the crops grew every year.

My parents continue to think what I do is exciting, whether it's color-coordinating a graph or sieving soil. Counting tulip identification and zipping her PJs among my daughter's achievements gives me some perspective on this, but that abiding interest and love is a great asset to me. Dick and Kim have welcomed me to consider the Wyoming Valley my home (no matter how many bean friends I bring along), and their interest in my work and family means the world to me. Dave has listened to me hash out every conceivable field or lab snafu, taken samples and toted our daughter around the field to keep me company, written code to munge the messiest data, and washed the dishes a lot, but still seems to be excited about me being a scientist. The rest of my friends and colleagues in Madison have made my time here a joy. Thank you.

Table of Contents

Chapter 1: What do we know about cover crop efficacy in the North Central U.S.?

1.1 Introduction

Recent meta-analyses about the efficacy of cover crops predict increased a) soil organic matter (Poeplau and Don 2015), b) arbuscular mycorrhizal fungi colonization (Bowles et al. 2017), and c) nutrient conservation (Quemada et al. 2013, Kladivko et al. 2014, Han et al. 2017), all while improving (with legume cover crops), or at least not reducing (with grass cover crops), main crop yields (Tonitto et al. 2006, Marcillo and Miguez 2017). These analyses broadly support cover crop adoption, and both survey and satellite data suggests that cover crop use in the North Central U.S. is increasing (Werblow 2015, Hamilton et al. 2017, Seifert et al. 2018). However, many studies show no benefit of cover crops (Poeplau and Don 2015, Han et al. 2017), and economic analyses show greater expenses and risks with cover crops (Pratt et al. 2014, Roth et al. 2018), so it is critical to clarify what costs and benefits may be expected in varying circumstances. Rather than expecting global benefits to mirror mean effect, we should take into account differences in cropping systems and climate to predict cover crop efficacy and set growers confidently on the lowest-risk path to success (Snapp et al. 2005, McLellen et al. 2018).

Here, we focus on the North Central U.S., a highly productive agricultural region with substantial constraints on cover cropping systems. First, we address establishment opportunities and constraints in the most common agricultural systems of the North Central U.S. to realistically depict cover crop systems in this area. Second, we evaluate the predicted benefits and costs of the typical cover crop scenarios. In conclusion, we use this information to re-frame approaches for promoting cover crops in the North Central U.S. in the hope that this will improve cover crop efficacy.

1.2 Establishment opportunities and constraints

In the northern U.S., options for establishing a successful cover crop are constrained by weather and prevailing crop rotations. Corn and soybean crops cover about 75% of agricultural land in the North

Central U.S. (US Department of Agriculture 2012). Cover crops are usually planted after fall harvest of cash crops, which provides a relatively small window for growth before the onset of cold temperatures. After harvest of corn grain and soybean, soil temperatures are only consistently favorable for germination and growth of winter rye (Figure 1, (Natural Resources Conservation Services (NRCS) n.d., USDA National Agricultural Statistics Service 2010)). While winter rye is effective at reducing nitrate losses and erosion, legume cover crops are more likely to increase yield of the following crop (Ruis et al. 2017, Marcillo and Miguez 2017, Gillette et al. 2018). More time for cover crop establishment is afforded by rotations that incorporate corn silage, vegetable processing crops, or a summer-harvested small grain like winter wheat, but wheat and silage represent a small fraction of cropland compared to corn and soybean (USDA National Agricultural Statistics Service 2010, US Department of Agriculture 2012, Seifert et al. 2018).

Poor establishment conditions and shorter cover crop growing seasons reduce cover crop productivity. Unsurprisingly, mean annual temperature and accumulated growing degree days were positively correlated to cover crop biomass production (Poeplau et al. 2015, Burger et al. 2017), and water availability and soil seedbed conditions dictated cover crop emergence (Constantin et al. 2015, Tribouillois et al. 2018). Strock et al. (2004) estimated that favorable conditions for establishing and growing rye after corn occurred only 25% of the time based on 41 years of weather data from Lamberton, MN. Farmers are already aware of this climatic reality: cover crop use in Michigan, Wisconsin and Minnesota lags behind that in Illinois, Indiana, and Ohio, and is less likely to persist for multiple years (Seifert et al. 2018).

In order to lengthen the cover crop growing season, cover crops may also be established in standing cash crops via broadcasting (aerial or high-clearance equipment) or drilling (during early crop growth) shade-tolerant cover crop species. Bich et al. (2014) and Blanco-Canqui et al. (2017) successfully drilled and established red clover, annual ryegrass, and a wheat/clover mix into corn at the V5 stage without

sacrificing corn yield. Late-season aerial seeding practices are still being refined and may require high-clearance equipment or hiring custom operators. Residue buildup under conservation tillage may limit seed-to-soil contact under broadcast seeding scenarios, making drilling a more effective option for establishment of cover crops (Bich et al. 2014).

1.3 Cover crop benefits

Globally, cover crop biomass production is the foundation for improving many ecosystem services. For example, Finney et al. (2016) showed that N retention, weed suppression, and main crop biomass production were proportional to cover crop biomass production and tissue C:N ratio. Greater cover crop biomass generally leads to lower nitrate concentrations in tile drainage (Strock et al. 2004, Kaspar et al. 2012), greater cover crop N retention (Lacey and Armstrong 2015, White et al. 2017), and higher soil N concentrations (Barel et al. 2018). Spring growth was particularly important for increasing mean weight diameter of soil aggregates, a metric of soil structure and erodibility (Ruis et al. 2017) and reducing nitrate leaching (Alonso-Ayuso et al. 2018). Biomass production by cover crops varies widely (Basche et al. 2014, Ruis et al. 2017, Blanco-Canqui et al. 2017, Marcillo and Miguez 2017), so it is likely that growers experience a wide variation in benefits.

The relationship between cover crop biomass and benefits varies by benefit, and is likely to plateau at intermediate levels of cover crop biomass (Figure 2, Finney et al. 2016). Erosion benefits may plateau at lower biomass levels than other benefits. Runoff decreases with cover crops in a variety of conditions and we assume that full surface coverage would maximize this benefit, although cover crop root morphology can also affect hydraulic conductivity (Wendt and Burwell 1985, Zhu et al. 1989, Martin and Cassel 1992, Blanco-Canqui et al. 2013, Yu et al. 2016). Greater cover crop biomass may be required to build soil organic matter: this effect is variable across studies, illustrating a high degree of site-specificity (Sainju et al. 2015, Basche et al. 2016, Ruis et al. 2017, Blanco-Canqui et al. 2017, Marcillo and Miguez

2017). When specifically assessing soil C, the positive effects of cover crops were evident in some within-field topographic positions, but not others (Ladoni et al. 2016, Beehler et al. 2017), or only in particulate organic C (Snapp and Surapur 2018). This variability likely stems from differences in extant soil C pools and the ability of cover crops to protect these pools which is a function of cover crop productivity (Beehler et al. 2017). Main-crop yield response to cover crop biomass depends on the cover crop species, and includes negative responses if cover crops outcompete the main crop for space, light, water, and/or N. (White et al. 2017, Marcillo and Miguez 2017).

Retention of N by cover crops, of critical importance to improving water quality in agricultural landscapes, is facilitated by uptake of residual N during the cover crop growing season, followed by slow release of this N during decomposition of cover crop residues (Lacey and Armstrong 2015). Depending on timing of release, this can be a source of N for the main crop (Gentry et al. 2013). In addition, increased ground cover slows runoff during spring and fall rain events compared to bare fallow systems, which maintains clay-associated organic matter in place and reduces losses of soil organic matter and P (Zhu et al. 1989, Rhoton et al. 2002, Yu et al. 2016). These functions begin to occur even with low levels of cover crop biomass (Schipanski et al. 2014, Finney et al. 2016). Water quality in natural systems is a high priority across the Midwest and the expense of removing nitrates from drinking water is a burden on many municipalities (Minnesota Pollution Control Agency 2014, Iowa State University 2017). Cover crops can be a cost-effective water quality improvement option, especially when used strategically to address areas with identified nitrate leaching within the recharge area of a well or with identified problems from runoff/erosion contributing excess nutrients to surface water (Thomas et al. 2014, Kladivko et al. 2014, Roley et al. 2016, Ward et al. 2018).

1.4 Cover crop costs

Cover crop expenses generally include seed, planting, termination, and any reduction in main crop profit. Expenses associated with cover crops may be offset by 1) cost-sharing from local, state, and/or federal agencies, 2) reducing the need for fertilizer by increasing the supply of N to the cash crop (although farmers do not always do reduce fertilizer N accordingly (Pratt et al. 2014)), 3) using cover crop biomass for animal forage, or 4) increasing the quantity of main-crop residue available for harvest (Pratt et al. 2014, Roth et al. 2018). Cost-sharing for cover crops has increased across the U.S. since 2008, with greatest gains in Indiana, and Ohio (Seifert et al. 2018). While economic analysis of agronomic benefits and soil improvements shows a positive mean return for producers, the chance of losing money on cover crops ranges from 0 (with legumes and legume mixtures) to 77% (with oilseed radish) (Pratt et al. 2014). This stems from the high variability in cover crop biomass production discussed above and is likely a primary concern to farmers.

An average decrease in leaching, or an increase in yield, may be less important to the farmer decision-making process than the risk of main-crop failure. Risk of reduced main-crop yield when using cover crops is protected by the Federal Crop Insurance Program, if termination occurs in accordance with NRCS policies, which vary regionally based on water availability. Although grazing or haying a cover crop was the only system in which growers experienced positive net returns on cover crops in Iowa (Plastina et al. 2018), using cover crops as forages may be restricted by cost-sharing regulations, which presents a tradeoff between flexible management and reduced risk (Natural Resources Conservation Services (NRCS) 2014, Bergtold et al. 2017). Advanced cover crop users are often interested in shorter-season main-crop cultivars, alternative cover crop species, and work on marginal farmland, and while management experiments may complicate the use of cost-sharing, farmers claim greater benefits in main crop yield and soil health (Basche and Roesch-McNally 2017). More research is needed to establish best management practices for grazing and haying cover crops, especially the minimum quantity of biomass preserved in-field to prevent erosion and reduce nitrate leaching (likely a moving target based

on soil, climate and cropping system, e.g., Johnson et al. 2014). Given the low profit margins for corn and soybean systems (U.S. Department of Agriculture 2018), it is critical that cost-sharing supports farmers who wish to use practices like cover crop interseeding and grazing to shift towards more resilient agroecosystems.

1.5 Conclusions: Reframing cover crop programs in the North Central U.S.

Incorporating cover crops into agroecosystems in the North Central U.S. should be predicated on site-specific recommendations and supported by cost-sharing from federal, state and local agencies. We have long relied on farmers to act as stewards of a treasured communal resource while extracting their livelihoods from that same resource – the soil. This tension leaves farmers with a dilemma: should they experiment with cover crops and diversified rotations in hopes of improving soil health? Or, should they seek to maximize profits and minimize risks? In addition, much of the prime farmland in the U.S. is not farmed by the owners, so a long-term perspective on improving soil is often lacking. Cover crops represent a management tradeoff with a time lag: the farmer incurs an immediate risk while society may benefit in the long term. Cost-sharing has the effect of increasing cover crop area (Gonzalez-Ramirez et al. 2015), and if society values clean water and soil health, it is critical that we continue to share the risk of cover crop production with the farmer. Other options for improving water quality such as wetland establishment and two-stage ditches can be more cost-effective in the long term (Roley et al. 2016), but cover crop adoption has lower immediate costs and keeps farmland in production, so that farmers become partners in conservation. Promotion of cover crops should be founded on creating conditions for maximizing cover crop growth (e.g., planting after early main-crop harvest or investing in early seeding). Benefits and risks will be significantly affected by climate and edaphic variability. Further research is needed to ascertain whether benefits accrue when biomass production is low, as well as the likelihood of experiencing cover crop benefits in each crop rotation under diverse weather conditions.

Finally, governmental programs incentivizing and supporting cover crop adoption are critical to maintain societal “buy-in” on practices intended to improve common resources.

1.6 Acknowledgments

This work was supported by the DOE-Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494) and the DOE OBP Office of Energy Efficiency and Renewable Energy (DE-AC05-76RL01830). Thank you to the members of the University of Wisconsin Cover Crop Team, who have provided many insights shaping the ideas expressed above.

1.7 References

Alonso-Ayuso, M., M. Quemada, M. Vanclooster, M. Ruiz-Ramos, A. Rodriguez, and J. L. Gabriel. 2018. Assessing cover crop management under actual and climate change conditions. Science of The Total Environment 621 (April 15, 2018):1330–1341.

Barel, J. M., T. W. Kuyper, W. de Boer, J. C. Douma, and G. B. De Deyn. 2018. Legacy effects of diversity in space and time driven by winter cover crop biomass and nitrogen concentration. Journal of Applied Ecology 55(1):299–310.

Basche, A. D., S. V. Archontoulis, T. C. Kaspar, D. B. Jaynes, T. B. Parkin, and F. E. Miguez. 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 (February 15, 2016):95–106.

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

Basche, A. D., and G. E. Roesch-McNally. 2017. Research topics to scale up cover crop use: Reflections from innovative Iowa farmers. Journal of Soil and Water Conservation 72(3):59A–63A.

Beehler, J., J. Fry, W. Negassa, and A. Kravchenko. 2017. Impact of cover crop on soil carbon accrual in topographically diverse terrain. Journal of Soil and Water Conservation (2017).

Bergtold, J. S., S. Ramsey, L. Maddy, and J. R. Williams. 2017. A review of economic considerations for cover crops as a conservation practice. Renewable Agriculture and Food Systems (May 31, 2017):1–15.

Bich, A. D., C. L. Reese, A. C. Kennedy, D. E. Clay, and S. A. Clay. 2014. Corn yield is not reduced by mid-season establishment of cover crops in northern great plains environments. Crop Management

13(1).

Blanco-Canqui, H., J. D. Holman, A. J. Schlegel, J. Tatarko, and T. M. Shaver. 2013. Replacing Fallow with Cover Crops in a Semiarid Soil: Effects on Soil Properties. Soil Science Society of America Journal 77(3):1026.

Blanco-Canqui, H., M. Sindelar, C. S. Wortmann, and G. Kreikemeier. 2017. Aerial interseeded cover crop and corn residue harvest: Soil and crop impacts. Agronomy Journal 109(4):1344–1351.

Bowles, T. M., L. E. Jackson, M. Loeher, and T. R. Cavagnaro. 2017. Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. Journal of Applied Ecology 54(6):1785–1793.

Burger, M., M. R. Dumlao, J. Wang, B. A. Moradi, W. R. Horwath, and W. K. Silk. 2017. Cover Crop Development Related to Nitrate Uptake and Cumulative Temperature. Crop Science 57(2):971.

Constantin, J., C. Dürr, H. Tribouillois, and E. Justes. 2015. Catch crop emergence success depends on weather and soil seedbed conditions in interaction with sowing date: A simulation study using the SIMPLE emergence model. Field Crops Research 176 (May 1, 2015):22–33.

Finney, D. M., C. M. White, and J. P. Kaye. 2016. Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. Agronomy Journal 108(1):39–52.

Gentry, L. E., S. S. Snapp, R. F. Price, and L. F. Gentry. 2013. Apparent red clover nitrogen credit to corn: evaluating cover crop introduction. Agronomy Journal 105(6):1658–1664.

Gillette, K., R. W. Malone, T. C. Kaspar, L. Ma, T. B. Parkin, D. B. Jaynes, Q. X. Fang, J. L. Hatfield, G. W. Feyereisen, and K. C. Kersebaum. 2018. N loss to drain flow and N2O emissions from a corn-soybean rotation with winter rye. Science of The Total Environment 618 (March 15, 2018):982–997.

Gonzalez-Ramirez, M. J., C. L. Kling, and J. G. J. Arbuckle. 2015. Cost-share Effectiveness in the Adoption of Cover Crops in Iowa. *In* Agricultural & Applied Economics Association and Western Agricultural Economics Association Annual Meeting. San Francisco, CA, USA.

Hamilton, A. V., D. A. Mortensen, and M. K. Allen. 2017. The state of the cover crop nation and how to set realistic future goals for the popular conservation practice. Journal of Soil and Water Conservation 72(5):111A–115A.

Han, Z., M. T. Walter, and L. E. Drinkwater. 2017. N2O 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.

Iowa State University. 2017. Iowa Nutrient Reduction Strategy Annual Progress Report. http://www.nutrientstrategy.iastate.edu/.

Johnson, J. M. F., J. M. Novak, G. E. Varvel, D. E. Stott, S. L. Osborne, D. L. Karlen, J. A. Lamb, J. Baker, and P. R. Adler. 2014. Crop residue mass needed to maintain soil organic carbon levels: can it be determined? BioEnergy Research 7(2):481–490.

Kaspar, T. C., D. B. Jaynes, T. B. Parkin, T. B. Moorman, and J. W. Singer. 2012. Effectiveness of oat and rye cover crops in reducing nitrate losses in drainage water. Agricultural Water Management 110 (July 1, 2012):25–33.

Kladivko, E. J., T. C. Kaspar, D. B. Jaynes, R. W. Malone, J. Singer, X. K. Morin, and T. Searchinger. 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.

Lacey, C., and S. Armstrong. 2015. The Efficacy of Winter Cover Crops to Stabilize Soil Inorganic Nitrogen after Fall-Applied Anhydrous Ammonia. Journal of Environment Quality 44(2):442.

Ladoni, M., A. Basir, P. G. Robertson, and A. N. Kravchenko. 2016. Scaling-up: cover crops differentially influence soil carbon in agricultural fields with diverse topography. Agriculture, Ecosystems & Environment 225 (June 2016):93–103.

Marcillo, G. S., and F. E. Miguez. 2017. Corn yield response to winter cover crops: An updated meta-analysis. Journal of Soil and Water Conservation 72(3):226–239.

Martin, C. K., and D. K. Cassel. 1992. Soil Loss and Silage Yield for Three Tillage Management Systems. Journal of Production Agriculture 5(4):581–586.

McLellen, E. L., K. E. Schilling, C. F. Wolter, M. D. Tomer, S. A. Porter, J. A. Magner, D. R. Smith, and L. S. Prokopy. 2018. Right practice, right place: A conservation planning toolbox for meeting water quality goals in the Corn Belt. Journal of Soil and Water Conservation2 73(2):29A–34A.

Minnesota Pollution Control Agency. 2014. Minnesota Nutrient Reduction Strategy. St Paul, Minnesota.

NRCS (Natural Resources Conservation Services (USDA)). Soil Climate Analysis Network Data and Products. https://www.wcc.nrcs.usda.gov/scan/.

NRCS, U. 2014. NRCS Cover Crop Termination Guidelines.

Plastina, A., F. Liu, W. Sawadgo, F. E. Miguez, S. Carlson, A. ; Plastina, F. E. ; Miguez, and G. Marcillo. 2018. Annual Net Returns to Cover Crops in Iowa. Ames, IA.

Poeplau, C., H. Aronsson, Å. Myrbeck, and T. Kätterer. 2015. Effect of perennial ryegrass cover crop on soil organic carbon stocks in southern Sweden. Geoderma Regional 4 (April 1, 2015):126–133.

Poeplau, C., and A. Don. 2015. Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. Agriculture, Ecosystems & Environment 200 (February 2015):33–41.

Pratt, M. R., W. E. Tyner, D. J. Muth, and E. J. Kladivko. 2014. Synergies between cover crops and corn

stover removal. Agricultural Systems 130 (September 1, 2014):67–76.

Quemada, M., M. Baranski, M. N. J. Nobel-de Lange, A. Vallejo, and J. M. Cooper. 2013. Meta-analysis of strategies to control nitrate leaching in irrigated agricultural systems and their effects on crop yield. Agriculture, Ecosystems and Environment 174 (July 15, 2013):1–10.

Rhoton, F. E., M. J. Shipitalo, and D. L. Lindbo. 2002. Runoff and soil loss from midwestern and southeastern US silt loam soils as affected by tillage practice and soil organic matter content. Soil and Tillage Research 66(1):1–11.

Roley, S. S., J. L. Tank, J. C. Tyndall, and J. D. Witter. 2016. How cost-effective are cover crops, wetlands, and two-stage ditches for nitrogen removal in the Mississippi River Basin? Water Resources and Economics 15 (July 1, 2016):43–56.

Roth, R. T., M. D. Ruffatti, P. D. O'Rourke, and S. D. Armstrong. 2018. A cost analysis approach to valuing cover crop environmental and nitrogen cycling benefits: A central Illinois on farm case study. Agricultural Systems 159 (January 1, 2018):69–77.

Ruis, S. J., H. Blanco-Canqui, P. J. Jasa, R. B. Ferguson, and G. Slater. 2017. Can cover crop use allow increased levels of corn residue removal for biofuel in irrigated and rainfed systems? Bioenergy Research 10(4):992–1004.

Sainju, U. M., B. A. Allen, T. Caesar-TonThat, and A. W. Lenssen. 2015. Dryland Soil Carbon and Nitrogen after Thirty Years of Tillage and Cropping Sequence Combination. Agronomy Journal 107(5):1822.

Schipanski, M. E., M. Barbercheck, M. R. Douglas, D. M. Finney, K. Haider, J. P. Kaye, A. R. Kemanian, D. A. Mortensen, M. R. Ryan, J. Tooker, and C. White. 2014. A framework for evaluating ecosystem services provided by cover crops in agroecosystems. Agricultural Systems 125 (March 1, 2014):12–22.

Seifert, C. A., G. Azzari, and D. B. Lobell. 2018. Satellite detection of cover crops and their effects on crop yield in the Midwestern United States. Environmental Research Letters (May 15, 2018).

Snapp, S. S., S. M. Swinton, R. Labarta, D. Mutch, J. R. Black, R. Leep, J. Nyiraneza, and K. O'Neil. 2005. Evaluating cover crops for benefits, costs and performance within cropping system niches. Agronomy Journal 97(1):322–332.

Snapp, S., and S. Surapur. 2018. Rye cover crop retains nitrogen and doesn't reduce corn yields. Soil and Tillage Research 180 (August 1, 2018):107–115.

Strock, J. S., P. M. Porter, and M. P. Russelle. 2004. Cover Cropping to Reduce Nitrate Loss through Subsurface Drainage in the Northern U.S. Corn Belt. Journal of Environment Quality 33(3):1010.

Thomas, C. A. J., M. J. Helmers, T. M. Isenhart, and J. Lawrence. 2014. Reducing Nutrient Loss: Science Shows What Works. Agriculture and Environment Extension Publications 223 (2014):1–4.

Tonitto, C., M. B. David, and L. E. Drinkwater. 2006. Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: A meta-analysis of crop yield and N dynamics. Agriculture, Ecosystems and Environment 112(1):58–72.

Tribouillois, H., J. Constantin, and E. Justes. 2018. Analysis and modeling of cover crop emergence: Accuracy of a static model and the dynamic STICS soil-crop model (2018).

USDA NASS. 2010. Field Crops Usual Planting and Harvesting Dates. Washington, D.C.

USDA NASS. 2012. Quick Stats. http://quickstats.nass.usda.gov/.

USDepartment of Agriculture. 2018. Commodity Costs and Returns. https://www.ers.usda.gov/data-products/commodity-costs-and-returns.aspx#historic1.

Ward, A., A. Sharpley, K. Miller, W. Dick, J. Hoorman, J. Fulton, and G. LaBarge. 2018. An assessment of

in-field nutrient best management practices for agricultural crop systems with subsurface drainage. Journal of Soil and Water Conservation2 73(1):5A–10A.

Wendt, R. C., and R. E. Burwell. 1985. Runoff and soil losses for conventional, reduced, and no-till corn. Journal of Soil and Water Conservation 40(5):450–454.

Werblow, S. 2015. 2014-2015 Annual Report Cover Crop Survey: A synopsis of the information collected during the 2014-2015 Cover Crop Survey.

White, C. M., S. T. DuPont, M. Hautau, D. Hartman, D. M. Finney, B. Bradley, J. C. LaChance, and J. P. Kaye. 2017. Managing the trade off between nitrogen supply and retention with cover crop mixtures. Agriculture, Ecosystems & Environment 237 (January 16, 2017):121–133.

Yu, Y., W. Loiskandl, H.-P. Kaul, M. Himmelbauer, W. Wei, L. Chen, and G. Bodner. 2016. Estimation of runoff mitigation by morphologically different cover crop root systems. Journal of Hydrology 538 (July 1, 2016):667–676.

Zhu, J. C., C. J. Gantzer, S. H. Anderson, E. E. Alberts, and P. R. Beuselinck. 1989. Runoff, Soil, and Dissolved Nutrient Losses from No-Till Soybean with Winter Cover Crops. Soil Science Society of America Journal 53(4):1210.

1.8 Figures

Figure 1. Minimum soil temperature at 0-5 cm (°C) during harvest period in top corn- and soybean-growing states. Vertical gray boxes represent the harvest period for most grain state-wide. Horizontal lines represent germination temperatures of clover (·····), brassica (- · - · -), and rye (– – –) cover crops (10, 7.2, and 1.1 °C). Temperature data represents a mean of all available years from the weather stations located at Mason, IL, Ames, IA, Crescent Lake, MN, Dexter, MO, and Rodgers Farm, NE (NRCS Soil and Climate Analysis Network, accessed 12/1/2017). Harvest period determined from USDA National Agricultural Statistics Service (2010).

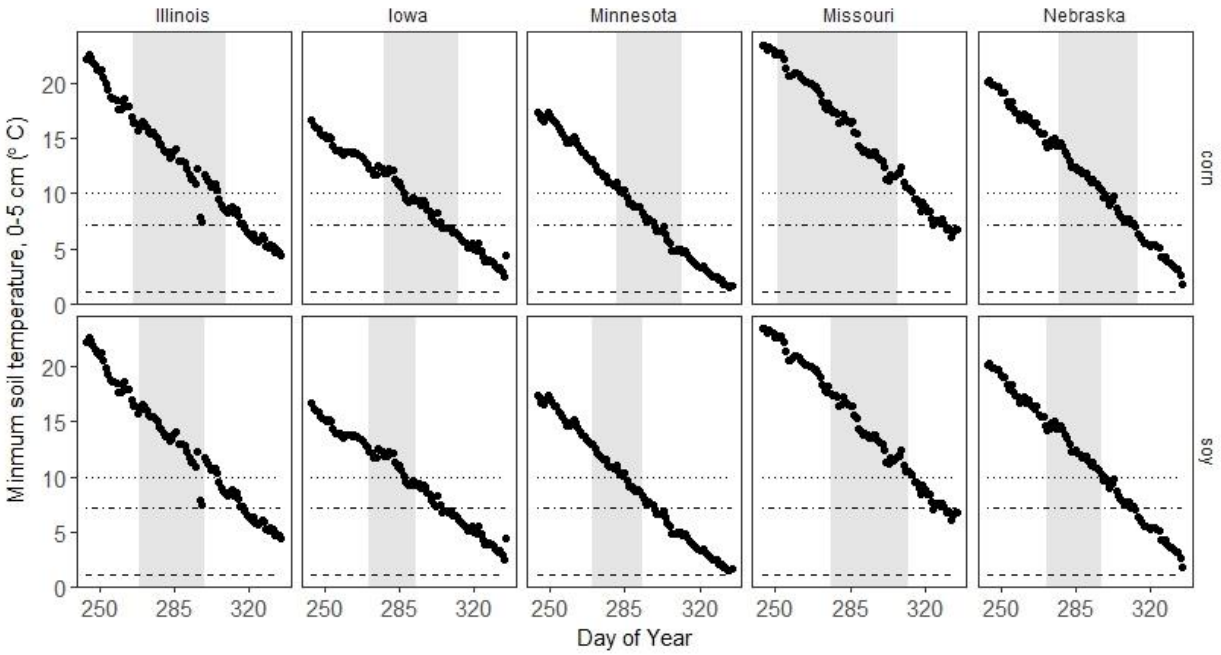
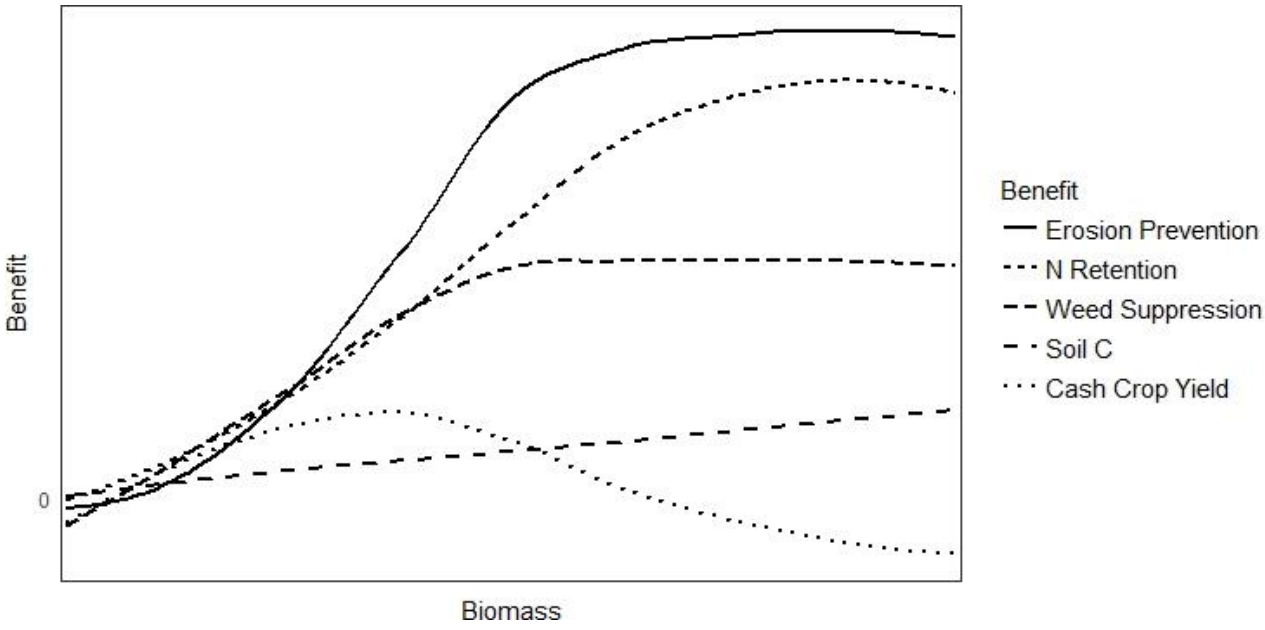


Figure 2. Conceptual diagram of how various environmental benefits accrue in relation to cover crop biomass, partially based on data from Finney et al. (2016) as well as literature review.



Chapter 2: Cover crop effects on net ecosystem carbon balance in grain and silage maize

Published in Agronomy Journal, *Crop Residue Workshop Special Issue* 2018. Doi: 10.2134/agronj2018.01.0045

2.1 Abstract

Cover crops have potential to increase net ecosystem C balance (NECB) and subsequent accrual of soil organic C (SOC) by lengthening the growing season in annual agriculture. By measuring net primary productivity (NPP) and C lost to harvest and heterotrophic respiration (R_h), our objective was to evaluate NECB of annual (winter rye, *Secale cereale* L.) and perennial (Kentucky bluegrass, *Poa pratensis* L.) cover crops compared to no cover crop control in continuous maize (*Zea mays* L.) harvested for either grain or silage. There was no effect of cover crop on NECB, but grain maize NECB was greater than silage (32 vs -433 g C m^{-2}), indicating greater SOC sink when maize residue was retained. Rye was more productive in silage (147 g C m^{-2}) compared to grain (32 g C m^{-2}). Rye increased total belowground NPP in silage maize (rye: 326, no cover: 275, bluegrass: 268 g C m^{-2}) but bluegrass decreased aboveground NPP in grain (rye: 1079, no cover: 1179, bluegrass: 1026 g C m^{-2}) and silage (rye: 1037, no cover: 1025, bluegrass: 864 g C m^{-2}). Yield was lower under bluegrass (781 g C m^{-2}) than no cover (962 g C m^{-2}) in silage. Losses of C to R_h varied by year, but not by harvest or cover crop. While cover crops may provide multiple benefits to farmers and society, their capacity to directly increase SOC may be low.

2.2 Introduction

The ability of agroecosystems to increase SOC depends on NECB–the difference between C fixed from the atmosphere into biomass (NPP) and C returned to the atmosphere as R_h or removed through

harvest. Positive NECB values indicate an ecosystem is a net C sink, while negative values indicate an ecosystem is a net C source to the atmosphere (Russell et al. 2009, Oates and Jackson 2014). Seasonally, sufficient precipitation and heat for productive crops also increase R_h, so C inputs and outputs from the system increase concurrently. However, agricultural management may also affect C fluxes, usually to the detriment of SOC accumulation. For example, tillage exposes SOC in soil aggregates to heterotrophic microbes, which stimulates R_h (Six et al. 1999, Grandy and Robertson 2006, Stewart et al. 2017). Fertilization with inorganic N can stimulate NPP, but it also alters the microbial community structure and sometimes increases decomposition of SOC (Dijkstra et al. 2005, Grandy et al. 2013, Oates et al. 2016). Harvesting maize residue for cellulosic bioenergy production or maize silage represents a potential loss of SOC because maize residue inputs can be critical for soil C (Liska et al. 2014, Johnson et al. 2014). Estimating NECB under various management scenarios allows for site-specific comparison of management effects on long-term C storage potential.

Agroecosystem NPP may be increased by growing cover crops outside the cash crop growing season (i.e. Hubbard et al., 2013). Moreover, cover crops may confer many benefits to farmers and the environment such as reduced nitrate leaching (Kladivko et al. 2014) and soil erosion (Blanco-Canqui et al. 2015), increased nutrient availability and crop yield (Piotrowska and Wilczewski 2012, Gentry et al. 2013, Maltas et al. 2013, Varela et al. 2014), and soil C sequestration (Sainju et al. 2002, Poeplau and Don 2015, Poeplau et al. 2015). Despite the evidence for environmental benefits, cover crops were planted on <30% of potentially available land in 2012, so barriers to adoption remain (Hamilton et al. 2017). Cover crops can be difficult to establish following grain maize harvest in the North Central U.S., leading to less cover crop biomass and other expected ecosystem services (Finney et al. 2016). Farmers in this region have adapted by planting cover crops following soybeans, wheat, and silage maize in rotation, and relying more heavily on winter cereal cover crops such as rye rather than leguminous cover crops. Research specific to these cropping systems, as well as technical assistance and more information on

successful cover crop use, may increase farmer interest and adoption (Arbuckle and Roesch-McNally 2015), with potential for widespread environmental benefits (reviewed by Blanco-Canqui et al., 2015).

The effects of cover crops on the NECB of agricultural systems was neutral in maize-soybean and wheat-soybean rotations (Baker and Griffis 2005, Gebremedhin et al. 2012), but has not been investigated with cover crops planted after maize. This is particularly relevant as interest in cellulosic bioenergy production increases, incentivizing more maize planting and residue harvest (Mehaffey et al. 2012, Lark et al. 2015). Cover crops may be able to help stabilize soil and provide organic C inputs to offset C lost to harvest (Pratt et al. 2014, Jones et al. 2018). Given low potential for SOC accumulation in annual agricultural rotations of the U.S. Corn Belt (Zeri et al. 2011, Sanford et al. 2012, Cates and Ruark 2017, Collier et al. 2017, Abraha et al. 2018), more research on whether cover crops increase the NECB of corn agroecosystems is needed. Multi-year studies of the NECB of cover crop systems are necessary to evaluate the conditions when cover crops have a positive impact on NECB and subsequently create conditions favorable for SOC accumulation. Land managers and policy makers can take this information into account when deciding where and how to incorporate and incentivize cover crops into maize systems.

We applied management practices common in the northern Corn Belt of the U.S. to rye and bluegrass cover crop systems for three years on a prairie-derived Mollisol in southern Wisconsin. Our objectives were to investigate a) how incorporation of a cover crop into no-till maize affects the NECB (including NPP, R_h, and yield) of the system and b) how harvest for grain maize compares to silage maize in NECB, crop yield, and cover crop success. We hypothesized that cover crops would increase NPP, harvested yield of grain or silage maize, and R_h, and that NECB would be greater when cover crops were used. We also hypothesized that silage maize would allow for greater cover crop establishment and productivity.

2.3 Materials and methods

2.3.1 Experimental design

Our plots were part of the Department of Energy-Great Lakes Bioenergy Research Center's Bioenergy Cropping Systems Experiment located at the Arlington Agricultural Research Station in Arlington, WI (43°17'45" N, 89°22'48" W and 315 m asl), previously described in Sanford et al. (2016). The predominant soil series is a Plano silt loam (fine-silty, mixed, superactive mesic typic Argiudolls), which developed from loess over glacial till. At 0 to 10 cm, SOC was 22 mg kg^{-1}, available P and K were 151 and 189 mg kg^{-1}, and exchangeable Ca and Mg were 1578 and 458 mg kg^{-1}. Soil profiles were characterized thoroughly in Sanford et al. (2016). The experiment was a randomized complete block split-plot design (n=5 blocks). Whole-plots (silage maize or grain maize) were 27 x 43 m (0.12 ha) with 12-m alleys between adjacent plots and split-plots (rye, bluegrass, or no cover crop) were 27 x 12.2 m (0.03 ha) with 3-m alleys between split plots. Thirty-year (1981 to 2010) mean annual precipitation was 869 mm and mean annual temperature was 6.9 °C. For study years 2015 to 2017, annual precipitation was 993, 987, and 855 mm and mean temperature was 8.1, 8.8 and 8.2 °C, respectively.

2.3.2 Crop management

Treatments were applied with "best management practices" for the crop in question, thus the timing of maize harvest, strip-tilling, and cover crop maintenance varied among treatments. Rye was drilled following maize harvest and terminated with glyphosate (Durango ® DMA ®, Dow AgroSciences, Indianapolis, IN) at 3.2 L ha^{-1} immediately prior to maize planting the following spring. Rye seeding rates were higher in grain maize than silage maize, to compensate for planting through residue, but varied based on planter operator each year (Table 1). Bluegrass was drill-seeded three times in the first 18 months of the study to establish the stand (Table 1). In 2016 and 2017, bluegrass was sprayed with a non-lethal dose of glufosinate-ammonium at maize planting to minimize competition with maize growth. In silage maize treatment, plots were strip-tilled after harvest in the fall. In grain maize

treatment, plots were strip-tilled prior to maize planting in the spring. The maize hybrid P0448R was drill-seeded into all plots in early May at 83,980 seeds ha^{-1}. Silage maize was harvested at ~60% moisture in mid-September while grain was harvested in late October. Ground cover for cover crops was estimated by evaluating photos taken from 1 m above the ground in three locations per plot, for the proportion of pixels that were green (Richardson et al. 2001). Success of cover crop establishment was determined by photographic analyses of cover each spring and varied by year and maize system (Table 1).

2.3.3 Estimating NPP

We estimated NPP by collecting biomass at crop physiological maturity as described in Sanford et al. (2016), or, in the case of rye, immediately before termination in early May. A 1-m^2 quadrat was placed randomly in each plot, and all aboveground biomass was clipped. Maize belowground biomass was removed to 15-cm depth within the 1-m^2 plot. Bluegrass and rye belowground biomass was estimated by taking five 5 × 15-cm deep root cores per plot. All root samples were kept cool until hand washing over a 0.5-mm sieve. Soil cores have been shown to capture two times the fine root biomass of ingrowth cores, increasing our confidence of fully representing bluegrass and rye BNPP (Ostonen et al. 2005). In bluegrass plots, root samples were collected throughout the year, while rye roots were only sampled at peak biomass. As perennial grass roots turn over throughout the growing season but some roots persist for multiple years (Fransen and De Kroon 2001), bluegrass annual root growth was estimated as the difference between peak root biomass and root biomass at the first spring sampling. All aboveground and root biomass was dried at 50 °C for 24 h, weighed, ground, and analyzed for C and N content, except a mean value of maize grain C (42.9 g g^{-1}) was used for all years and all samples. Adjustments to measured BNPP were made based on literature values: root exudation as 42.9% of ANPP in cover crops (Austin et al. 2017) and 11% of total NPP in maize (Jones et al. 2009); root turnover at 53% in cover crops based on an estimate for grasslands (Gill & Jackson 2000); and all BNPP was corrected for depth

based on an assumption that 66% of all roots were found at the measured depth of 15 cm (Jackson et al. 1996).

2.3.4 Estimating R_s

We used infra-red gas analyzers (IRGAs, LiCor 6400-09 soil CO_2 flux chamber, Lincoln, NE) to estimate R_s about twice monthly in periods when soil was not frozen (March through November). Soil temperature and moisture at 15-cm depth were measured at the same time using a temperature probe and time domain reflectrometry, respectively (FieldScout TDR 350, Aurora, IL). Chambers consisted of 5-cm tall, 10.2-cm inner-diameter polyvinyl chloride collars inserted 2 cm into the soil between maize rows to maximize representation of the different cover crop environments. Living cover crops were folded when possible and clipped if necessary (Collier et al. 2016). Two or three R_s measurements per plot were made within a 24-h period between 10:00 and 16:00. While diurnal variation in R_s can be significant at this site (±20% of the daily mean in June, von Haden, unpublished data), 75% of our measurements took place between 10:00 and 13:00, when R_s variation was <10%. Overall, our measurement timing may have overestimated R_s, so we included corrections for diurnal variability for months where some site-specific estimates of Rs variability were available (von Haden 2017). Corrected calculations did not reduce variability in the cumulative R_s or qualitatively shift interpretation of results so uncorrected R_s estimates were used since we did not have diurnal corrections for many of the months in which we sampled.

We estimated R_h accounting for phenological development of maize and cover crops. For example, prior to maize planting R_h/R_s was assumed to be 1 in the no cover treatment since no plants were present. In bluegrass and rye prior to maize planting, R_h/R_s was assumed to gradually decrease from 0.9 to 0.7 because of increased photosynthesis rates over the same period, not quite reaching the minimum R_h/R_s of 0.5 described in wheat (Suleau et al. 2011, Zhang et al. 2013) because of glyphosate application and maize planting prior to maximum cover crop productivity. These assumptions were informed by root-

exclusion estimates of R_h/R_s on maize and switchgrass plots in 2015 and 2016 at the same site (von Haden 2017), taking into account lower productivity of cover crops compared to mature switchgrass stands. All cover crop treatments were assumed to have the same R_h/R_s between maize canopy closure and harvest, presuming that rapid maize growth dominated autotrophic respiration.

Cumulative R_h was estimated by linear interpolation between measurements, because temperature and moisture measurements did not predict R_h rates and linear interpolation is the best simple algorithm for gap-filling R_s (Gomez-Casanovas et al. 2013). Linear interpolation likely neglects peaks in R_s occurring after fertilization, tillage disturbance, and moisture spikes, but we assumed these effects were similar across treatments, and evidence shows that twice monthly sampling likely is within 10% of the best estimate of R_s (Savage et al. 2008). Testing the effect of the strongest possible positive bias on R_h, we found that if cumulative R_h was decreased 10%, effects of cover crop and maize harvest were the same. Overall NECB was greater when R_h was decreased 10% but NECB values with a sign change, indicating a switch from C source to C sink, were not significantly different from 0. Given the low confidence we have in estimating bias in R_h because of seasonal changes in diurnal variation, and lack of significant effect on interpretation, we have elected to report uncorrected means. Rates of R_h were compared in three seasons (pre-plant, maize growing, and post-harvest) determined by the maize planting and harvesting date for each system and year to evaluate how the effects of cover crops on R_h rates varied seasonally.

2.3.5 Calculating NECB

Net ecosystem carbon balance is different than net ecosystem production (NEP) in that it incorporates management factors such as harvested biomass removal to the carbon balance. We calculated NECB as:

$$NECB = NPP - (R_h + Harvest) \quad \text{(Eq 1)}$$

Where NPP is the sum of above- and below-ground NPP as described above, R_h is cumulative heterotrophic respiration, and harvest is biomass removed at maize harvest. These calculations assumed that NPP represented all C fixed into plant biomass. Note that we represent NPP as positive, and R_h and harvest as negative, so a positive NECB signifies the system is a net sink of C and a negative NECB signifies a net source of C to the atmosphere.

2.3.6 Statistical analyses

The effects of year, cover crops, and harvest treatments on ground cover, ANPP, BNPP, cumulative R_h, maize harvest, and NECB were analyzed as fixed effects in a mixed effects model with block and treatment × block as random effects. For R_h rates, season replaced year as a fixed effect. If a significant effect of treatment was found ($P<0.05$), separation of treatments by least squares means is presented. Where interactions were significant, we focused on the effects of harvest or treatment within a year. All analyses were conducted in R including the package *emmeans* (R Core Team 2016).

2.4 Results

Cover crop ground cover was greater under silage maize compared to grain maize ($P<0.0001$, Table 1), but there was an interaction between harvest and cover treatment in total cover NPP (Figure 1, Table 2). Rye NPP was greater under silage maize than grain (147.4 vs. 38.2 g C m^{-2}, $P<0.0001$) while bluegrass NPP did not differ between harvest treatments (average bluegrass NPP 83.5 g C m^{-2}, $P=0.25$, Table 2, Figure 1). After 2015, rye was more productive than bluegrass under silage, but bluegrass was always more productive under grain ($P<0.0001$). Maize dominated total productivity: cover NPP averaged 13% of total NPP in rye with silage maize, 2% in rye with grain maize, 8% in bluegrass with silage maize and 6% in bluegrass with grain maize (Figure 1).

Grain maize had significantly increased NECB compared to silage maize (31.7 vs -434 g C m^{-2}, Tables 2 and 3). Significant interactions between year, harvest, and cover crop treatments on NECB resulted from

annual variability in ANPP and BNPP, as well as yield and total R_h (Table 2). In grain maize, bluegrass increased NECB compared to rye in 2016 and no cover increased NECB compared to bluegrass in 2015 (Table 3). In silage maize, there was no effect of cover crop treatment on NECB, despite five-fold greater rye NPP in silage than grain (Figure 1). Maize harvest treatment did not affect ANPP or BNPP. Bluegrass depressed ANPP in 2015, and rye on average increased BNPP relative to no cover in silage (Table 3).

Silage maize yield was higher than grain maize yield across cover treatments and years (Tables 2 and 3). Silage maize yield was significantly lower in bluegrass than no cover across years and in 2015 (Table 3). We suspect that the non-lethal quantity of herbicide sprayed on bluegrass at maize planting in 2016 and 2017 mitigated the 2015 yield penalty imposed by the intercropped bluegrass. Silage maize yield with rye did not significantly differ from no cover in any year, though it was generally slightly lower. Grain yield did not differ by cover treatment, although grain yield with bluegrass was generally lower than no cover.

Cumulative R_h only differed among cover treatments in 2016 silage maize (Table 3), when losses were greater in rye than bluegrass, but there were significant interactions between harvest, cover and time of year for R_h rate (Table 4). In silage maize, R_h rate was greater under both cover crops than no cover during the pre-plant period and maize growing season (Table 4, $P<0.0001$). No differences in R_h rate were observed among cover treatments in grain maize. The highest R_h rates were observed during the maize growing season in all years and both harvest treatments, concurrent with the highest soil temperatures of the year (Figure 2, Table 4, $P<0.0001$). Soil temperature was not different among cover treatments but was greater in silage maize in the postharvest period, likely because the postharvest period began earlier for silage maize. Soil moisture varied within and among growing seasons, and there was no significant difference among treatments (data not shown).

2.5 Discussion

We observed a tradeoff between cover crop productivity and NECB – cover crops were most productive when maize was harvested as silage but removing most of the maize plant for silage made these agroecosystems C sources to the atmosphere. A compromise may ensue whereby cover crop growers harvest only a portion of silage maize or incorporate shorter-season grain maize hybrids or small grains into rotations. However, cover crop productivity may be unpredictable and highly variable even under ideal conditions due to the short growing seasons in the northern U.S (Strock et al. 2004). In our study, rye NPP was in the range 1.3 to 430 g C m^{-2}, and in a three-year Nebraska study rye biomass was in the range 0.023 to 3.35 Mg ha^{-1} (Ruis et al. 2017).

High biomass variability leads to variation in cover crop effects on NECB and subsequent SOC accumulation. Increases in cover crop biomass have been shown to increase weed suppression, N retention, and N availability, but decrease cash-crop yield (Finney et al. 2016). This mix of costs and benefits makes it difficult for growers to predict risk in relation to potential soil C benefits of cover crops. Factors that appear to reduce risk include early seeding, early termination, using a mixture of species including clover, and using the cover crop as forage for livestock (Kramberger et al. 2014, Kladivko et al. 2014, Bich et al. 2014, Arbuckle and Roesch-McNally 2015, Blanco-Canqui et al. 2017). Iowa growers claimed that increased education and funding for initial cover crop experiments would increase adoption, but until growers are confident that specific cover management and species mixtures are low-risk in their systems, adoption likely will be slow (Arbuckle and Roesch-McNally 2015, Basche and Roesch-McNally 2017).

We anticipated that R_h would increase under cover crops because they can stimulate the soil microbial respiration via root exudates – a process known as rhizosphere priming (Kuzyakov 2010, Shahzad et al. 2015, Austin et al. 2017). Rhizosphere priming may have been the cause of higher R_h rates relative to control when cover crops were growing prior to maize planting. In contrast, decomposition of dead cover crop biomass may have been the cause of higher rates of R_h during the maize growing season, and

this source of C was likely consumed by the maize harvest. Austin et al. (2017), found that five months after termination, new C added from cover crops had declined substantially. Despite seasonal increases in R_h rates, cover crops did not increase cumulative R_h, indicating that increased mineralization of SOM by rhizosphere priming was not a significant portion of NECB. In addition, the quantity of rhizodeposition is correlated to biomass production, so priming will be contingent on cover productivity, which is highly variable as discussed above (Kuzyakov and Cheng 2001, Cheng et al. 2003).

Our data indicated that SOC was not likely to accumulate with cover crop use, despite other work showing that cover crops increase SOC (Poeplau and Don 2015, Jones et al. 2018). However, increasing SOC is a slow process, and it is possible that if cover crops remain in place, NECB may become more favorable for SOC accrual. The mechanism for this may be accumulation of mineral-associated microbial C during cover crop growth, which offsets seasonal priming of native SOC decomposition (Grandy and Neff 2008, Kallenbach et al. 2015, Mbuthia et al. 2015, Austin et al. 2017). Maize cropping is known to have neutral and often negative NECB, which has led to the long-term decline in SOC across the U.S. Corn Belt (Baker and Griffis 2005, Joo et al. 2016, von Haden 2017, Sanderman et al. 2017). Practices such as cover cropping may slowly increase NECB, but annual and spatial variation is such that some years may be negative, as in our study, and small changes will be difficult to detect (Skinner 2008, Sanderman and Baldock 2010, Muñoz et al. 2014, Beehler et al. 2017, Tifafi et al. 2018). However, 75% of cover crop users claim soil health benefits after less than three years of the practice, suggesting that either cover crops are accruing soil benefits on-farm that we did not observe, or that farmers' perceptions of soil improvements outpace changes in SOC (Conservation Technology Information Center/Sustainable Agriculture Research and Education, 2017).

The silage maize treatment employed here can inform our understanding of residue removal for cellulosic bioenergy, which must take into account site-specific NECB and the C footprint of biofuel production, as well as environmental impacts of the likely alternative, managing the residue in place.

Guidelines for crop reside removal based on soil health could be useful to growers, industry, and regulators, but evidence suggests precipitation, temperature, soil properties, and tillage regimen will condition residue removal recommendations (Johnson et al. 2014). To maintain agricultural systems as C-neutral, or even as C sinks, residue should be removed only when NECB is positive, and only to the point when NECB is zero. Although our study occurred on a single site, the fact that NECB remained near zero, even when residue was retained, reinforces Johnson et al.'s (2014) conclusions that residue removal rates should be determined at spatial resolutions equivalent to the field level, and that annual variation will require flexible, adaptive management. In addition, leaving residue on the surface leads to cooler spring soil temperatures, and to avoid delayed maize emergence growers may strip till or apply fall N to speed decomposition of surface residue, although this has not been shown to be effective (Al-Kaisi and Guzman 2013).

2.6 Conclusions

Bluegrass and no cover crop tended to increase the NECB under grain maize, but NECB of all grain treatments remained near zero, indicating a neutral C balance of these agroecosystems. Under silage maize, NECB was always negative, indicating a source of C to the atmosphere, and NECB in silage was not affected by cover crops despite higher rye ground cover and NPP. On these productive Mollisols, maize NPP dominated the total C inputs to the agroecosystem, so any residue or silage removal must take annual variation in maize productivity into account. Cover crop stimulation of SOC mineralization increased R_h losses seasonally, but not cumulatively. To maintain a positive NECB (net C sink) and increase SOC on Corn Belt Mollisols, growing grain maize and retaining residue appears to be more effective than planting cover crops. However, long-term dynamics of cover crop systems are not well understood, and the trend towards decreased R_h losses over the three-year study suggests that over time, cover crops may have a positive impact on NECB. In the short term, while cover crop users may

experience many benefits such as erosion prevention and reduced nutrient leaching, their capacity to directly increase soil C may be low.

2.7 Acknowledgments

The authors are grateful to Gary Oates, Gregg Sanford, Alex Butz, Mark Walsh, Jimmy Sustachek, Casey Menick, Sarah Lee and Kelsey Johnson for field and laboratory assistance. Anonymous reviewers helped to improve the final manuscript. Funding was provided in part by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DEFC0207ER64494) and DOE OBP Office of Energy Efficiency and Renewable Energy (DEAC0576RL01830).

2.8 References

Abraha, M., S.K. Hamilton, J. Chen, and G.P. Robertson. 2018. Ecosystem carbon exchange on conversion of Conservation Reserve Program grasslands to annual and perennial cropping systems. Agric. For. Meteorol. 253–254: 151–160.

Al-Kaisi, M.M., and J.G. Guzman. 2013. Effects of tillage and nitrogen rates on decomposition of transgenic Bt and near-isogenic non-Bt maize residue. Soil Tillage Res. 129: 32–39.

Arbuckle, J.G., and G. Roesch-McNally. 2015. Cover crop adoption in Iowa: The role of perceived practice characteristics. J. Soil Water Conserv. 70(6): 418–429.

Austin, E.E., K. Wickings, M.D. McDaniel, G.P. Robertson, and A.S. Grandy. 2017. Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. GCB Bioenergy 9(7): 1252–1263.

Baker, J.M., and T.J. Griffis. 2005. Examining strategies to improve the carbon balance of corn/soybean agriculture using eddy covariance and mass balance techniques. Agric. For. Meteorol. 128(3–4): 163–177.

Basche, A.D., and G.E. Roesch-McNally. 2017. Research topics to scale up cover crop use: Reflections from innovative Iowa farmers. J. Soil Water Conserv. 72(3): 59A–63A.

Beehler, J., J. Fry, W. Negassa, and A. Kravchenko. 2017. Impact of cover crop on soil carbon accrual in topographically diverse terrain. J. Soil Water Conserv.

Bich, A.D., C.L. Reese, A.C. Kennedy, D.E. Clay, and S.A. Clay. 2014. Corn yield is not reduced by mid-season establishment of cover crops in northern great plains environments. Crop Manag. 13(1).

Blanco-Canqui, H., T.M. Shaver, J.L. Lindquist, C.A. Shapiro, R.W. Elmore, C.A. Francis, and G.W. Hergert. 2015. Cover crops and ecosystem services: insights from studies in temperate soils. Agron. J. 107(6): 2449–2474.

Blanco-Canqui, H., M. Sindelar, C.S. Wortmann, and G. Kreikemeier. 2017. Aerial interseeded cover crop and corn residue harvest: Soil and crop impacts. Agron. J. 109(4): 1344–1351.

Cates, A.M.A.M., and M.D.M.D. Ruark. 2017. Soil aggregate and particulate C and N under corn rotations: responses to management and correlations with yield. Plant Soil 415(1–2): 1–13.

Cheng, W., D.W. Johnson, and S. Fu. 2003. Rhizosphere effects on decomposition. Soil Sci. Soc. Am. J. 67(5): 1418–1427.

Collier, S.M., A.P. Dean, L.G. Oates, M.D. Ruark, and R.D. Jackson. 2016. Does plant biomass manipulation in static chambers affect nitrous oxide emissions from soils? J. Environ. Qual. 45(2): 751–756.

Collier, S.M., M.D. Ruark, M.R. Naber, T.W. Andraski, and M.D. Casler. 2017. Apparent stability and subtle change in surface and subsurface soil carbon and nitrogen under a long-term fertilizer gradient. Soil Sci. Soc. Am. J. 81(2): 310–321.

Conservation Technology Information Center/Sustainable Agriculture Research and Education, and . 2017. Cover Crop Survey.

Dijkstra, F.A., S.E. Hobbie, P.B. Reich, and J.M.H. Knops. 2005. Divergent effects of elevated CO2, N fertilization, and plant diversity on soil C and N dynamics in a grassland field experiment. Plant Soil 272(1–2): 41–52.

Finney, D.M., C.M. White, and J.P. Kaye. 2016. Biomass production and carbon/nitrogen ratio influence ecosystem services from cover crop mixtures. Agron. J. 108(1): 39–52.

Fransen, B., and H. De Kroon. 2001. Long-term disadvantages of selective root placement: root proliferation and shoot biomass of two perennial grass species in a 2-year experiment. J. Ecol. 89(5): 711–722.

Gebremedhin, M.T., H.W. Loescher, and T.D. Tsegaye. 2012. Carbon balance of no-till soybean with winter wheat cover crop in the Southeastern United States. Agron. J. 104(5): 1321–1335.

Gentry, L.E., S.S. Snapp, R.F. Price, and L.F. Gentry. 2013. Apparent red clover nitrogen credit to corn: evaluating cover crop introduction. Agron. J. 105(6): 1658–1664.

Gill, R.A., and R.B. Jackson. 2003. Global patterns of root turnover for terrestrial ecosystems. New Phytol. 147(1): 13–31.

Gomez-Casanovas, N., K. Anderson-Teixeira, M. Zeri, C.J. Bernacchi, and E.H. DeLucia. 2013. Gap filling strategies and error in estimating annual soil respiration. Glob. Chang. Biol. 19(6): 1941–1952.

Grandy, A.S., and J.C. Neff. 2008. Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. Sci. Total Environ. 404(2–3): 297–307.

Grandy, A.S., and G.P. Robertson. 2006. Aggregation and organic matter protection following tillage of a previously uncultivated soil. Soil Sci. Soc. Am. J. 70(4): 1398–1406.

Grandy, A.S., D.S. Salam, K. Wickings, M.D. McDaniel, S.W. Culman, and S.S. Snapp. 2013. Soil respiration and litter decomposition responses to nitrogen fertilization rate in no-till corn systems. Agric. Ecosyst. Environ. 179: 35–40.

von Haden, A.C. 2017. Mechanisms of ecosystem carbon storage and stability in temperate bioenergy cropping systems.

Hamilton, A. V., D.A. Mortensen, and M.K. Allen. 2017. The state of the cover crop nation and how to set realistic future goals for the popular conservation practice. J. Soil Water Conserv. 72(5): 111A–115A.

Hubbard, R.K., T.C. Strickland, and S. Phatak. 2013. Effects of cover crop systems on soil physical properties and carbon/nitrogen relationships in the coastal plain of southeastern USA. Soil Tillage Res. 126: 276–283.

Jackson, R.. B., J. Canadell, J.R. Ehleringer, H.A. Mooney, O.E. Sala, E.D.D. Schulze, H.A. Ehleringer, O.E. Mooney, and E.D.D. Schulze. 1996. A global analysis of root distributions for terrestrial biomes. Oecologia 108(3): 389–411.

Johnson, J.M.F., J.M. Novak, G.E. Varvel, D.E. Stott, S.L. Osborne, D.L. Karlen, J.A. Lamb, J. Baker, and P.R. Adler. 2014. Crop residue mass needed to maintain soil organic carbon levels: can it be determined? BioEnergy Res. 7(2): 481–490.

Jones, D.L., C. Nguyen, and R.D. Finlay. 2009. Carbon flow in the rhizosphere: carbon trading at the soil–root interface. Plant Soil 321(1–2): 5–33.

Jones, C.D., L.G. Oates, G.P. Robertson, and R.C. Izaurralde. 2018. Perennialization and Cover Cropping Mitigate Soil Carbon Loss from Residue Harvesting. J. Environ. Qual. 47(0): 1–8.

Joo, E., M.Z. Hussain, M. Zeri, M.D. Masters, J.N. Miller, N. Gomez-Casanovas, E.H. DeLucia, and C.J. Bernacchi. 2016. The influence of drought and heat stress on long-term carbon fluxes of bioenergy crops grown in the Midwestern USA. Plant. Cell Environ. 39(9): 1928–1940.

Kallenbach, C.M., A.S. Grandy, S.D. Frey, and A.F. Diefendorf. 2015. Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biol. Biochem. 91: 279–290.

Kladivko, E.J., T.C. Kaspar, D.B. Jaynes, R.W. Malone, J. Singer, X.K. Morin, and T. Searchinger. 2014. Cover crops in the upper midwestern United States: Potential adoption and reduction of nitrate leaching in the Mississippi River Basin. J. Soil Water Conserv. 69(4): 279–291.

Kramberger, B., A. Gselman, J. Kristl, M. Lešnik, V. Šuštar, M. Muršec, and M. Podvršnik. 2014. Winter cover crop: The effects of grass-clover mixture proportion and biomass management on maize and the apparent residual N in the soil. Eur. J. Agron. 55: 63–71.

Kuzyakov, Y. 2010. Priming effects: Interactions between living and dead organic matter. Soil Biol. Biochem. 42(9): 1363–1371.

Kuzyakov, Y., and W. Cheng. 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biol. Biochem. 33(14): 1915–1925.

Lark, T.J., J. Meghan Salmon, and H.K. Gibbs. 2015. Cropland expansion outpaces agricultural and biofuel policies in the United States. Environ. Res. Lett. 10(4): 044003.

Liska, A.J., H. Yang, M. Milner, S. Goddard, H. Blanco-Canqui, M.P. Pelton, X.X. Fang, H. Zhu, and A.E. Suyker. 2014. Biofuels from crop residue can reduce soil carbon and increase CO2 emissions. Nat. Clim. Chang. 4(5): 398–401.

Maltas, A., R. Charles, B. Jeangros, and S. Sinaj. 2013. Effect of organic fertilizers and reduced-tillage on soil properties, crop nitrogen response and crop yield: Results of a 12-year experiment in Changins, Switzerland. Soil Tillage Res. 126: 11–18.

Mbuthia, L.W., V. Acosta-Martínez, J. DeBruyn, S. Schaeffer, D. Tyler, E. Odoi, M. Mpheshea, F. Walker, N. Eash, J. DeBryun, S. Schaeffer, D. Tyler, E. Odoi, M. Mpheshea, F. Walker, and N. Eash. 2015. Long term tillage, cover crop, and fertilization effects on microbial community

structure, activity: Implications for soil quality. Soil Biol. Biochem. 89: 24–34.

Mehaffey, M., E. Smith, and R. Van Remortel. 2012. Midwest U.S. landscape change to 2020 driven by biofuel mandates. Ecol. Appl. 22(1): 8–19.

Muñoz, J.D., J.P. Steibel, S. Snapp, and A.N. Kravchenko. 2014. Cover crop effect on corn growth and yield as influenced by topography. Agric. Ecosyst. Environ. 189: 229–239.

Oates, L.G., D.S. Duncan, G.R. Sanford, C. Liang, and R.D. Jackson. 2016. Bioenergy cropping systems that incorporate native grasses stimulate growth of plant-associated soil microbes in the absence of nitrogen fertilization. Agric. Ecosyst. Environ. 233: 396–403.

Oates, L.G., and R.D. Jackson. 2014. Livestock management strategy affects net ecosystem carbon balance of subhumid pasture. Rangel. Ecol. Manag. 67(1): 19–29.

Ostonen, I., K. Lõhmus, and K. Pajuste. 2005. Fine root biomass, production and its proportion of NPP in a fertile middle-aged Norway spruce forest: Comparison of soil core and ingrowth core methods. For. Ecol. Manage. 212(1–3): 264–277.

Piotrowska, A., and E. Wilczewski. 2012. Effects of catch crops cultivated for green manure and mineral nitrogen fertilization on soil enzyme activities and chemical properties. Geoderma 189–190: 72–80.

Poeplau, C., H. Aronsson, Å. Myrbeck, and T. Kätterer. 2015. Effect of perennial ryegrass cover crop on soil organic carbon stocks in southern Sweden. Geoderma Reg. 4: 126–133.

Poeplau, C., and A. Don. 2015. Carbon sequestration in agricultural soils via cultivation of cover crops – A meta-analysis. Agric. Ecosyst. Environ. 200: 33–41.

Pratt, M.R., W.E. Tyner, D.J. Muth, and E.J. Kladivko. 2014. Synergies between cover crops and corn stover removal. Agric. Syst. 130: 67–76.

R Core Team. 2016. R: a Language and Environment for Statistical Computing.

Richardson, M.D., D.E. Karcher, and L.C. Purcell. 2001. Quantifying Turfgrass Cover Using Digital Image Analysis. Crop Sci. 41(6): 1884.

Ruis, S.J., H. Blanco-Canqui, P.J. Jasa, R.B. Ferguson, and G. Slater. 2017. Can cover crop use allow increased levels of corn residue removal for biofuel in irrigated and rainfed systems? Bioenergy Res. 10(4): 992–1004.

Russell, A.E., C.A. Cambardella, D.A. Laird, D.B. Jaynes, and D.W. Meek. 2009. Nitrogen fertilizer effects on soil carbon balances in Midwestern U.S. agricultural systems. Ecol. Appl. 19(5): 1102–1113.

Sainju, U.., B.. Singh, and W.. Whitehead. 2002. Long-term effects of tillage, cover crops, and nitrogen fertilization on organic carbon and nitrogen concentrations in sandy loam soils in Georgia, USA. Soil Tillage Res. 63(3–4): 167–179.

Sanderman, J., and J.A. Baldock. 2010. Accounting for soil carbon sequestration in national inventories: a soil scientist's perspective. Environ. Res. Lett. 5(3): 034003.

Sanderman, J., T. Hengl, and G.J. Fiske. 2017. Soil carbon debt of 12,000 years of human land use. Proc. Natl. Acad. Sci. U. S. A. 114(36): 9575–9580.

Sanford, G.R., L.G. Oates, P. Jasrotia, K.D. Thelen, G.P. Robertson, and R.D. Jackson. 2016. Comparative productivity of alternative cellulosic bioenergy cropping systems in the North Central USA. Agric. Ecosyst. Environ. 216: 344–355.

Sanford, G.R., J.L. Posner, R.D. Jackson, C.J. Kucharik, J.L. Hedtcke, and T.L. Lin. 2012. Soil carbon lost from Mollisols of the North Central U.S.A. with 20 years of agricultural best management practices. Agric. Ecosyst. Environ. 162: 68–76.

Savage, K., E.A. Davidson, and A.D. Richardson. 2008. A conceptual and practical approach to data quality and analysis procedures for high-frequency soil respiration measurements. Funct. Ecol. 22(6): 1000–1007.

Shahzad, T., C. Chenu, P. Genet, S. Barot, N. Perveen, C. Mougin, and S. Fontaine. 2015. Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. Soil Biol. Biochem. 80: 146–155.

Six, J., E.T. Elliott, and K. Paustian. 1999. Aggregate and soil organic matter dynamics under conventional and no-tillage systems. Soil Sci. Soc. Am. J. 63(5): 1350–1358.

Skinner, R.H. 2008. High biomass removal limits carbon sequestration potential of mature temperate pastures. J. Environ. Qual. 37(4): 1319–26.

Stewart, C.E., A.D. Halvorson, and J.A. Delgado. 2017. Long-term N fertilization and conservation tillage practices conserve surface but not profile SOC stocks under semi-arid irrigated corn. Soil Tillage Res. 171: 9–18.

Strock, J.S., P.M. Porter, and M.P. Russelle. 2004. Cover Cropping to Reduce Nitrate Loss through Subsurface Drainage in the Northern U.S. Corn Belt. J. Environ. Qual. 33(3): 1010.

Suleau, M., C. Moureaux, D. Dufranne, P. Buysse, B. Bodson, J.-P. Destain, B. Heinesch, A. Debacq, and M. Aubinet. 2011. Respiration of three Belgian crops: Partitioning of total ecosystem respiration in its heterotrophic, above- and below-ground autotrophic components. Agric. For. Meteorol. 151(5): 633–643.

Tifafi, M., B. Guenet, and C. Hatté. 2018. Large Differences in Global and Regional Total Soil Carbon Stock Estimates Based on SoilGrids, HWSD, and NCSCD: Intercomparison and Evaluation Based on Field Data From USA, England, Wales, and France. Global Biogeochem. Cycles 32(1): 42–56.

Varela, M.F., C.M. Scianca, M.A. Taboada, and G. Rubio. 2014. Cover crop effects on soybean residue decomposition and P release in no-tillage systems of Argentina. Soil Tillage Res. 143: 59–66.

Zeri, M., K. Anderson-Teixeira, G. Hickman, M. Masters, E. DeLucia, and C.J. Bernacchi. 2011. Carbon exchange by establishing biofuel crops in Central Illinois. Agric. Ecosyst. Environ. 144(1): 319–329.

Zhang, Q., H.-M. Lei, and D.-W. Yang. 2013. Seasonal variations in soil respiration, heterotrophic respiration and autotrophic respiration of a wheat and maize rotation cropland in the North China Plain. Agric. For. Meteorol. 180: 34–43.

2.9 Tables and Figures

Figure 1. Mean net primary production (SE, n=5) of maize and cover crops, including above- and below-ground productivity.

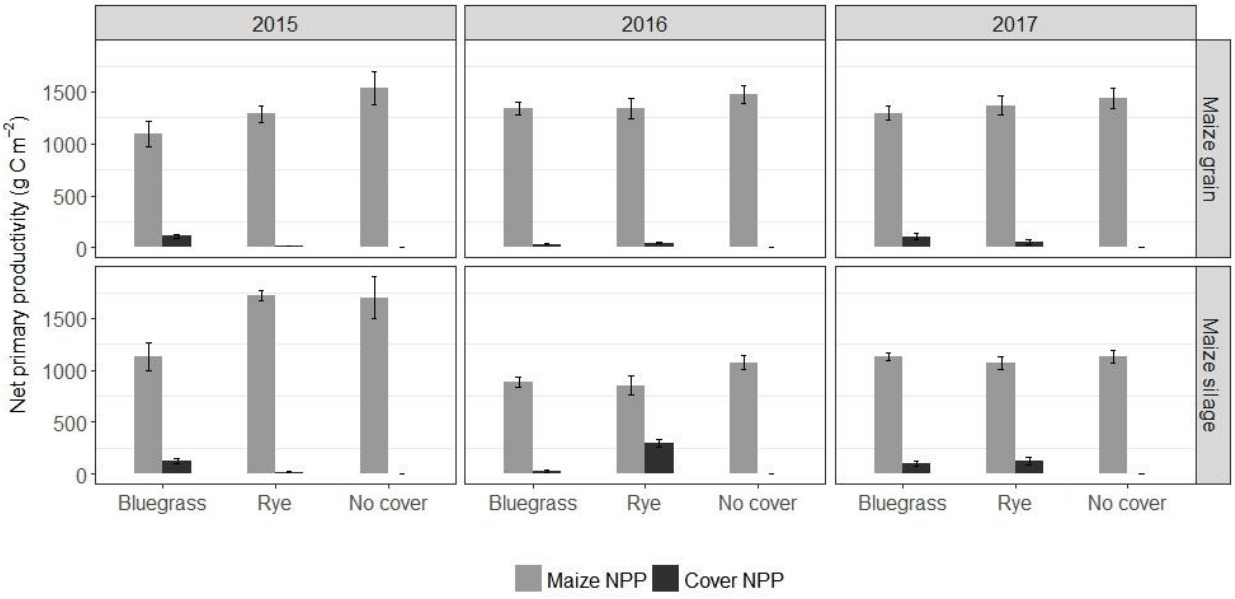


Figure 2. Heterotrophic respiration (R_h) response to soil temperature 2015 through 2017. Each point represents the mean of 3 to 5 plots. Pre-plant measurements took place from time of first thaw (late March) to maize planting in early May. Maize harvest took place in mid-September (Maize silage) or late October (Maize grain) and post-harvest measurements continued until the soil froze in early December.

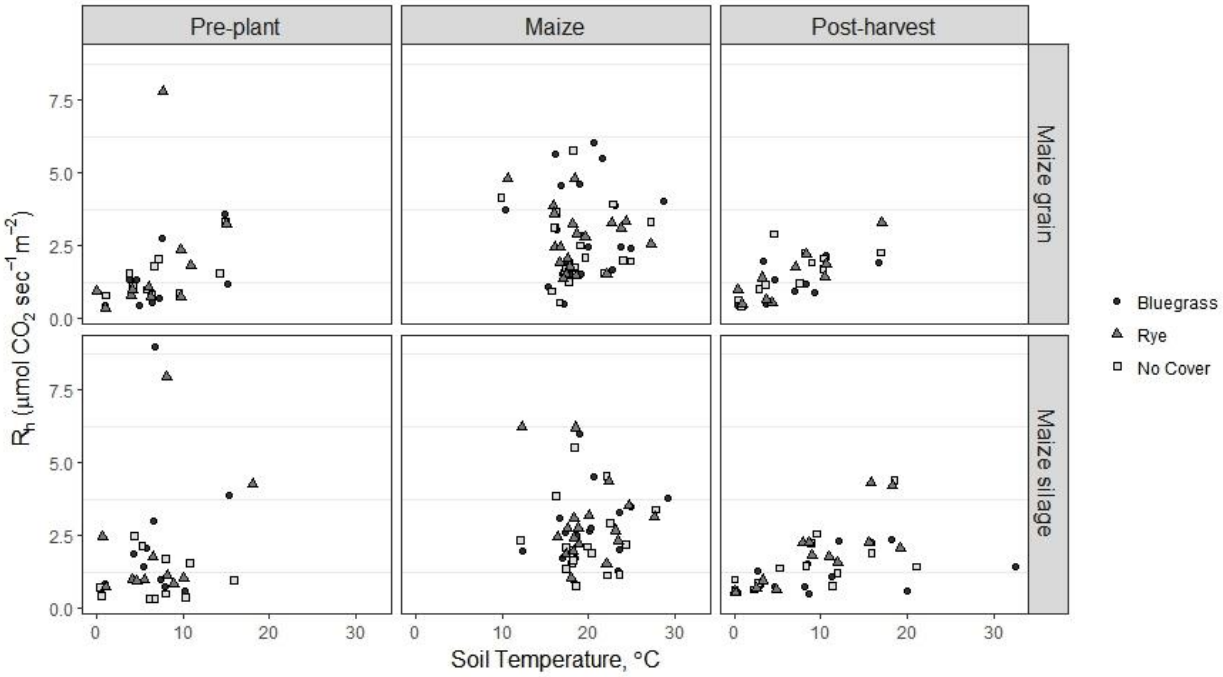


Table 1: Cover crop establishment and success. Note that ground cover data was only collected in 2016 and 2017, and that bluegrass was not planted annually after establishment 2014-2015.

		Grain maize			Silage maize		
Year	Cover crop	Seeding Date	Seeding Rate (kg ha^{-1})	Ground cover in spring (%)	Seeding Date	Seeding Rate (kg ha^{-1})	Ground cover in spring (%)
2015	Bluegrass	5/19/2014	22	NA	5/19/2014	22	NA
		11/3/2014	20	NA	11/3/2014	20	NA
		4/6/2015	17	NA	4/6/2015	17	NA
	Rye	10/22/2014	95	NA	10/22/2014	95	NA
2016	Bluegrass	–	–	13.0 (3.3)	–	–	21.1 (3.8)
	Rye	10/14/2015	155	33.3 (2.0)	9/24/2015	112	83.6 (2.9)
2017	Bluegrass	–	–	5.3 (0.6)	–	–	12.9 (2.1)
	Rye	10/14/16	118	39.0 (2.0)	9/15/2016	100	63.4 (3.9)

Table 2: ANOVA P-values for net ecosystem C balance (NECB) and various components: total aboveground net primary productivity (ANPP), total belowground net primary productivity (BNPP), above- and belowground cover crop net primary productivity (cover NPP) harvested biomass (Harvest), and cumulative annual heterotrophic soil respiration (R_h). Harvest treatments are maize grain and maize sileage, cover treatments are rye, bluegrass, and no cover, and years were 2015, 2016, and 2017.

Treatment	ANPP	BNPP	Cover NPP	Yield	R_h	NECB
			P-value			
Year (Y)	<0.001	<0.0001	<0.05	<0.0001	<0.0001	<0.0001
Harvest (H)	0.11	0.65	<0.01	<0.001	0.94	<0.001
Cover (C)	<0.001	0.06	<0.0001	<0.01	0.89	0.75
Y*H	<0.0001	<0.05	<0.0001	<0.0001	0.90	<0.01
Y*C	<0.05	0.09	<0.0001	<0.001	<0.01	<0.05
H*C	0.14	<0.01	<0.0001	0.28	0.42	0.16
Y*H*C	0.58	0.42	<0.0001	0.06	0.89	0.50

Table 3. Means (standard errors) for NECB and various components (): Aboveground net primary productivity (ANPP), belowground net primary productivity (BNPP) (includes both maize and cover crop biomass C), harvested biomass (Yield), and cumulative annual heterotrophic soil respiration (R_h). Lower case letters indicate differences among cover treatments within a year and harvest treatment (*P<0.05)*.

Year / Cover	Grain maize					Silage maize				
	ANPP	BNPP	Yield	R_h	NECB	ANPP	BNPP	Yield	R_h	NECB
	g C m^{-2}									
2015										
Bluegrass	910 (107) b	288 (25)	-398 (40)	-1127 (79)	-327 (88) b	965 (99) b	293 (21)	-859 (110) a	-1112 (74)	-705 (86)
Rye	1029 (57) ab	274 (23)	-571 (18)	-894 (84)	-163 (120) ab	1393 (47) a	347 (5)	-1249 (47) b	-959 (47)	-468 (50)
No cover	1220 (126) a	317 (32)	-539 (9)	-1039 (57)	-41 (186) a	1367 (173) a	332 (31)	-1308 (173) b	-987 (43)	-596 (44)
2016										
Bluegrass	1104 (56)	271 (11)	-427 (9)	-671 (13) b	277 (79) a	712 (34)	202 (11) b	-663 (36)	-726 (108)	-476 (99)
Rye	1108 (84)	268 (15)	-448 (14)	-931 (148) a	3.5 (85) b	830 (66)	318 (27) a	-628 (73)	-946 (47)	-426 (61)
No cover	1194 (69)	280 (14)	-461 (2)	-768 (102) ab	245 (61) ab	858 (57)	214 (12) b	-800 (58)	-743 (102)	-470 (98)
2017										
Bluegrass	1065 (49)	335 (8)	-620 (45)	-612 (37)	167 (50)	916 (29	310 (14)	-831 (28)	-687 (53)	-290 (67)
Rye	1099 (76)	323 (26)	-649 (47)	-668 (29)	104 (45)	888 (40)	312 (19)	-750 (43)	-632 (21)	-181 (52)
No cover	1124 (23)	313 (23)	-667 (38)	-752 (43)	19 (45)	850 (40)	281 (22)	-777 (43)	-646 (35)	-292 (48)
Mean										
Bluegrass	1026 (80) b	298 (20)	-481 (56)	-803 (116)	39 (140)	864 (77) b	268 (26) b	-781 (75) a	-842 (117)	-490 (111)
Rye	1079 (69) ab	288 (23)	-556 (48)	-831 (107)	-18 (97)	1037 (127) a	326 (19) a	-876 (134) ab	-792 (91)	-358 (79)
No cover	1179 (88) a	303 (24)	-556 (45)	-853 (90)	74 (122)	1025 (150) a	275 (31) b	-962 (151) b	-846 (79)	-453 (85)

Table 4. Mean heterotrophic respiration (R_h) rates (SE) by season and treatment 2015 through 2017. Lowercase letters indicate differences among cover crop treatments within season and harvest treatment (P<0.05). Uppercase letters indicate a difference between harvest treatments within cover crop and season (*P<0.05)*

Season	Cover	Grain maize		Silage maize		Both harvest treatments	
		R_h (µmol CO_2 sec^{-1})					
Pre-plant	Bluegrass	0.90 (0.10)		1.30 (0.21)	a	1.09 (0.11)	ab
	Rye	1.24 (0.24)		1.60 (0.19)	a	1.41 (0.16)	a
	No cover	1.02 (0.11)		0.88 (0.17)	b	0.96 (0.10)	b
Maize season	Bluegrass	2.51 (0.11)	B	2.81 (0.12)	aA	2.65 (0.08)	a
	Rye	2.57 (0.10)		2.91 (0.10)	a	2.74 (0.07)	a
	No cover	2.25 (0.10)		2.15 (0.10)	b	2.20 (0.08)	b
Post-Harvest	Bluegrass	1.19 (0.16)		1.18 (0.12)		1.29 (0.10)	
	Rye	1.54 (0.12)		1.64 (0.15)		1.59 (0.10)	
	No cover	1.44 (0.13)		1.40 (0.13)		1.42 (0.09)	
Full season	Bluegrass	1.91 (0.08)		2.08 (0.09)	a	1.98 (0.06)	b
	Rye	2.07 (0.09)		2.35 (0.08)	a	2.21 (0.06)	a
	No cover	1.84 (0.07)		1.74 (0.08)	b	1.79 (0.05)	b

Chapter 3: Cover crop effects on key components of the C cycle in maize cropping systems

3.1 Abstract

Cover crops are touted for their ability to improve many ecosystem services provided by annual cropping systems. In addition to water and nutrient retention, cover crops may influence C cycling by increasing total C inputs to the agroecosystem, stimulating microbial populations, or altering the rate of main crop residue decomposition. We assessed whether annual (rye) or perennial (bluegrass) cover crops in maize cropping systems influenced maize residue decomposition (litterbags) or microbial communities (shotgun metagenomics) in soil and litter, and whether these cover crops had an effect on microbially active pools of C, particulate organic matter (POM) C and N, or potentially mineralizable C (PMC). Neither cover crop affected litterbag decay rates or microbial composition relative to no cover crop controls. However, both cover crop types increased PMC indicating that microbially-available C was boosted by off-season C inputs from cover crops. Also, total POM and POM-N was higher with bluegrass cover crops, which indicates potential for greater C stabilization over time. The modest effects of cover crops on soil C pools suggest that their promotion should focus on soil protection and nutrient retention benefits rather than climate stabilization.

3.2 Introduction

Cover crops have been reported to increase soil organic C (SOC, Poeplau and Don 2015) but not to increase overall net ecosystem exchange (Baker and Griffis, 2005; Gebremedhin et al., 2012). This discrepancy likely stems from the myriad and conflicting effects that cover crops may have on components of the C cycle and subsequent SOC stabilization in annual agroecosystems. Cover crops can have a positive effect on SOC by a) increasing total C inputs through addition of litter (Austin et al., 2017), b) increasing active microbial pools of C (Kallenbach et al., 2015), and/or c) decreasing

decomposition rate of main crop residue. Alternatively, they can have a negative effect on SOC by priming decomposition of native SOC (Zhu et al., 2014) and/or increasing the decomposition rate of main crop residue (Varela et al., 2014). Depending on the season, topography, and crop rotation, one of these processes may dominate the response of SOC to cover crops, leading to the wide variation seen in SOC response to cover crops (Muñoz et al. 2014; Poeplau and Don 2015).

Root exudates from the growing cover crop may increase decomposition of native SOC through rhizosphere priming (Dijkstra et al., 2009; Zhu et al., 2014). In addition to increasing the loss of native soil C, rhizosphere priming may increase microbial biomass and shift the composition, and perhaps the function, of microbial communities (Cheng, 2009). On the other hand, cover crop root exudates may themselves constitute a substantial input of bioavailable C and stimulate growth of microbial biomass C (Austin et al., 2017), which may be the basis for some stable SOC (Kallenbach and Grandy, 2015). Measurable C pools such as potentially mineralizable C (PMC) and particulate organic matter (POM), considered early indicators of the direction of SOC change resulting from agronomic management, have been shown to increase under cover crops, perhaps because of increasing microbial activity (Ladoni et al., 2016; McDaniel et al., 2014; Snapp and Surapur, 2018).

When cover crops are terminated, the addition of cover crop biomass represents a pulse of C into the system if left to decompose in place. The decomposition of this litter will result in leaching of soluble C compounds through the soil profile and transformation of litter fragments into POM (Cotrufo et al., 2015). Depending on the soil type and disturbance level, a small quantity of cover crop C may be physically incorporated in soil aggregates and sorbed on mineral surfaces, leading to long-term SOC stabilization (Austin et al., 2017).

By shifting temperature and moisture patterns on the surface, living or dead cover crops may affect the decomposition of main crop residues, which are the majority of the total C input to the agroecosystem

(Cates and Jackson, 2018; Chen et al., 2018; Dijkstra et al., 2010, 2009; Flerchinger et al., 2003; Varela et al., 2014). In addition, there is the potential for cover crops to alter mesofauna composition, altering the decomposer abundance, community, and pathway for residue decomposition (Blubaugh et al., 2016; Leslie et al., 2017). Microbial composition and extracellular enzyme activity (EEA) may signal shifts in residue decomposition processes (Carreiro et al., 2000; Keiblinger et al., 2012). Maize residue decomposition rate is of particular interest in northern climates, where concerns over spring soil temperatures prompt growers to harvest, incorporate, or fertilize maize residue in an attempt to speed decomposition, despite limited efficacy (Al-Kaisi et al., 2017).

In a previous study, we evaluated the effects of rye (annual grass) and bluegrass (perennial grass) cover crops on net ecosystem C balance (NECB) of continuous maize and found no difference in NECB with cover crops compared to no cover, indicating cover crops did not increase C inputs relative to C losses (Cates and Jackson, 2018). In the same study, bluegrass and rye both increased early-season rates of heterotrophic respiration, suggesting that rhizosphere priming occurred with cover crops and raising questions about how cover crops may stimulate microbial mineralization of soil C. Here, we evaluated whether SOC accumulation under cover crops, via slower decomposition of maize residue and accumulation of microbially-available C, may be possible despite limited C inputs from cover crop litter. We measured decomposition of maize residue, metrics of labile C (PMC and POM), and SOC stocks after three years of rye or bluegrass cover crops in a continuous maize cropping system. To characterize decomposition with and without cover crops, we evaluated litter chemistry, microbial composition, and EEA in surface litter and adjacent soil. Building on the previous work, we also explored correlations between C inputs and active C metrics to illuminate what the C sources of these metrics might be. We hypothesized that cover crops would increase labile C metrics, alter microbial composition in soil and litter, and increase decomposition rate of surface residue.

3.3 Materials and methods

3.3.1 Site description

We conducted this study at the US DOE-Great Lakes Bioenergy Research Center's *Biomass Cropping Systems Experiment*, a randomized complete block (5 blocks), split-split plot (2 main-crop harvest, 3 cover crop treatments) design at the Arlington Agricultural Research Station near Arlington, WI (43°17'45"N, 89°22'48"W, 315 m a.s.l.). The soil is a Plano silt-loam, a well-drained Mollisol, mean annual temperature is 6.8 °C, and mean annual precipitation is 869 mm. A strip-till maize/cover crop trial began in 2014, with maize harvest (silage or grain) as a whole-plot treatment and cover crop species (rye, bluegrass, or no cover) as a split-plot treatment (described in Cates and Jackson 2018). Rye was drill-seeded each fall after maize harvest and killed with herbicide at maize planting. Bluegrass was drill-seeded in spring 2014, fall 2014, and spring 2015 and remained in perennial strips between maize rows thereafter. Plots were strip-tilled prior to maize planting. Whole plots were 27.4 x 42.7 m, and subplots were 27.4 x 12.2 m, with 3-m alleys between the cover crop treatments. Soil respiration measurements and samples of above- and below-ground net primary productivity (ANPP and BNPP) of cover and maize crops were collected 2015 through 2017, analyzed for C content, and reported in Cates and Jackson (2018).

3.3.2 Litterbag study

We performed a litterbag decomposition study between December 2015 and June 2017. Litterbags were fabricated with nylon mesh (50 μm openings for soil surface contact and 1.7-mm openings on the exposed upper bag to allow mesofauna access to litter). A total of 15 litterbags were placed in each plot every 0.5 m on an E-W transect. Each litterbag was 12 x 15 cm and filled with 10 g of maize stover (mixed stems and leaves). Maize stover was harvested in fall 2014 from adjacent plots, chopped into ~2-cm pieces, mixed thoroughly, and oven-dried. Litterbags were placed on the soil surface soil and fixed firmly in place with landscaping staples. Field operations avoided litterbags.

Two bags were collected from each plot at 18, 26, 34, 52, 71, and 82 weeks after placement, covering two winter and one summer seasons. The first bag was dried at 55 °C. Mass loss was determined as the difference between the initial mass and the dried mass, corrected for contamination by ashing a subsample in a muffle furnace. A subsample of dried litter was ground and analyzed for C and N using a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy). At 26 and 82 weeks, litter chemistry was assessed on dried ground litter using pyrolysis-gas chromatography/mass spectrometry (py-GC/MS). Litter organic C can be differentiated at various stages of decomposition using py-GC/MS (Grandy et al., 2009; Kallenbach and Grandy, 2015; Wickings and Grandy, 2013). Litter was pyrolized for 20 s at 600 °C, then transferred and separated on a GC over 60 min. An ion trap mass spectrometer detected peaks, which were then analyzed using the Automated Mass Spectral Deconvolution and Identification System (AMDIS, V 2.65) and the National Institute of Standards and Technology (NIST) compound library. Known C compounds were classified as aromatics, lipids, lignin, phenols, polysaccharides, N-bearing compounds or proteins and reported as percentages, based on each compound's peak area relative to total peak area of all identified peaks within a sample.

At each field collection, a second bag was split on site, with half used to assess EEA and half preserved for DNA extraction. The EEA sample was transported on ice to the lab, where it was immediately analyzed using a fluorometric technique (Saiya-Cork et al., 2002; Steinweg et al., 2012). Enzyme-specific fluorescent substrate was mixed with a slurry of sample and incubated for 3 h to allow enzymes to encounter the substrate. Fluorescence was measured using a microplate fluorometer with 365-nm excitation and 450-nm emission filters, using standard curves developed separately for each sample to account for absorbance. The activities of common hydrolytic enzymes critical to decomposition were measured at each sample date: α-glucosidase, β-glucosidase, β-D-cellobiosidase, L-Leucine-7-amidomethylcoumarin, N-acetyl-β-glucosidase, phosphatase, and β-xylosidase (AG, BG, CELL, LAP, NAG, PHOS, and XYL). To calculate the total enzyme efficiency, the activity of all enzymes was summed and

divided by the amount of C lost from litterbags at the final 82-week sample (Kallenbach and Grandy, 2015).

The subsample designated for DNA extraction (after 18, 26, 52, 71, 82 weeks) was transported from the field on dry ice, lyophilized, ground, and frozen at -80 °C until DNA was extracted using MoBio PowerEasy kits. Metagenomes were sequenced and phylogenetic assignments made for each contig by determining the lowest common ancestor according to the IMG NR database at the US DOE-Joint Genome Institute. Prior to analysis, we removed any assignments that were present in only one sample, rarefied to a consistent quantity of assignments per sample, and calculated the relative abundance of each phylogenetic assignment within a sample.

Two 2-cm dia x 5-cm deep soil cores were collected from below each litterbag at the time of collection, homogenized and treated identically to litter samples, except that EEA samples were refrigerated for 24 h prior to analysis.

3.3.3 Soil C pools

The PMC and POM are two metrics of microbially available soil C. The PMC is the C mineralized in a short-term incubation under ideal conditions and represents both the capacity of the microbial community and the quantity of readily available C substrate. The POM is the large organic material, mostly plant-derived, representing the future food source for detritivores and microbes.

Three 7.5-cm soil cores per subplot were collected in November 2017, representing the cumulative effect of three years of cover cropping. Cores were split into 0 to 10- and 10 to 25-cm sections, immediately weighed for moisture determination, and dried at 55 °C. The POM was determined as described in Cates et al. (2016). Briefly, 10.0 g soil was soaked in sodium hexametaphosphate overnight, shaken to completely destabilize the structure, and material >53 µm was retained as POM. The POM was homogenized and ground for C and N analysis as described above.

The PMC represents the flush of CO_2 during a 24-h incubation and was determined on 10.0 g of soil rewetted to 50% water-filled pore space and incubated in sealed 1-L canning jars at 25 °C (method adapted from Franzluebbers et al., 2000; Hurisso et al., 2016). The [CO_2} was measured using an infrared gas analyzer (LiCor LI-820, Lincoln, NE), and corrected for the [CO_2] of a blank (empty jar) incubated under the same conditions.

3.3.4 Statistical analysis

All statistical analyses were completed in R (R Core Team, 2016). Linear models of soil C and N were used to evaluate the effect of decomposition time on soil nutrient content below litterbags, but exponential models were applied to litter mass, C loss, and N loss based on expectations from previous work (e.g., Zhang et al. 2015; Mazzilli et al. 2015).

Individual enzyme activities and relative abundances of C compounds found in soil and litter were first evaluated using a mixed model (*aov* function) with treatment and date as fixed effects and block x treatment as random effects. However, block effects were not significant, and because of damage and loss of litterbags in the field, treatment and block samples sizes were uneven. Hence, block effects were dropped from litterbag enzyme models. Means comparisons were performed with Tukey's HSD test to evaluate significance of differences among treatments (P<0.05, *emmeans* package). The relative abundances of compound classes found in py-GC/MS and OTUs identified in metagenomes were visualized using non-metric multidimensional scaling (NMDS, function *metaMDS* in the R package vegan) and differences among treatments were evaluated using PERMANOVA (*adonis* in the R package vegan). To evaluate which phyla were most different among treatments, we conducted a Simper test on phyla identified in the metagenomes. Mantel tests were used to examine similarities between soil and litter microbial communities as well as between genomic and chemical data (*mantel* in the R package vegan).

The POM and PMC data were analyzed using a mixed model with depth, harvest, and cover treatments as fixed effects and block x treatment as random effects, with significant differences among treatments determined by means comparison with Tukey's HSD. Correlations between POM, PMC, and C inputs (total and cover crop ANPP and BNPP as well as all residue inputs) were assessed using the *cor.test* function in R.

3.4 Results

3.4.1 Litterbag decomposition, chemistry, and microbial composition

Contrary to our hypothesis, the decomposition rate and chemistry of maize litter was not affected by cover crop presence. The exponential decay rate rate of mass C and N loss did not differ among cover treatments (Table 1), although exponential decay models fit all data (Supplemental Figure 1). Litter chemistry was significantly different at 26 weeks than 82 weeks (PERMANOVA $P<0.01$, Figure 1a). Aromatics, lipids, phenols, and proteins were increased over time, while lignin and polysaccharides decreased (see supplemental material). No individual compounds or overall litter chemistry were affected by cover crop treatment.

Litter microbial activity, as evaluated by EEA, and microbial metagenome community composition differed over time but not by cover crop treatment. Individual EEA were highly variable over time (see supplemental material) but all cover crop treatments had the same total EEA efficiency after 18 months of litter decomposition (Table 1). Clusters in NMDS revealed that litter microbial communities were similar during the first spring (18 and 26 weeks), and during fall and second spring samples (52, 71 and 82 weeks, Figure 1b). PERMANOVA confirmed a significant effect of date ($P<0.01$) with no treatment effect for microbial composition. The two clusters differed in abundances of bacterial phyla, but not fungal or archaeal abundances. In particular, Bacteroidetes were correlated to the difference between early and later decomposition dates, and Proteobacteria differed among the later dates as well

according to SIMPER. In addition, a Mantel test showed a significant correlation between litter chemistry and microbial community at 26 and 82 weeks (r^2=0.17, *P*<0.01).

Chemical and microbial assays of soil immediately below litterbags also revealed significant change over the course of decomposition but few differences by cover crop treatment. Soil C and N increased over time (Figure 2a-b). Soil C chemistry broadly differed by date (PERMANOVA *P*<0.01, Figure 2c) and some compounds changed by cover crop treatment (see supplemental material). For example, rye increased relative abundances of polysaccharides but decreased proteins. Soil microbial composition was drastically different at 18 and 26 weeks than 52, 71 and 82 weeks (PERMANOVA *P*<0.01, Figure 2d). Proteobacteria were most correlated to differences among time points according to SIMPER. The soil microbial community was not correlated to soil C chemistry according to a Mantel test. Soil EEA varied by date, but not by cover crop (see supplemental material).

3.4.2 Total SOC and active C metrics

The SOC stocks did not differ among cover crop or harvest treatments, while cover crops broadly increased PMC and only bluegrass increased POM, providing partial support for our hypothesis that cover crops would increase active C. There was a significant interaction between cover and harvest type at both depths in PMC. Rye PMC was greater than no cover PMC in all harvest treatments and depths (*P*<0.05, Table 2). Bluegrass PMC was greater than no cover under silage harvest only (*P*<0.001, Table2). Total POM was also greater in bluegrass than no cover under silage, and POM-N was greater in bluegrass than rye under grain (*P*<0.05, Table2).

The C in total ANPP, BNPP and residue left on the soil were positively correlated with some active C metrics but cover NPP was not correlated with any active C metrics (Figure 3a-b). Total POM was not correlated with any active C metrics (data not shown), but POM-C and POM-N concentrations were significantly correlated with total residue C at both depths, largely driven by the larger residue C inputs

in grain harvest systems (566 vs 104 g C m^{-2} yr^{-1} in silage). However, PMC was greater in grain than silage harvest systems (P<0.0001, both depths), while POM-C, POM-N, and POM did not differ by harvest. In addition, total ANPP was correlated with PMC 0-10 cm and POM-C and POM-N 10 to 25 cm. Total BNPP was significantly correlated with PMC at both depths.

3.5. Discussion

Evidence for whether cover crops improve potential for SOC accumulation in maize was mixed. Microbially-available C in the form of POM and PMC was stimulated by cover crops, but SOC stocks, maize residue decomposition rates, and soil and litter microbial composition were not affected in our experiment. The drivers of above- and below-ground C dynamics as well as implications of our findings for building SOC through cover crops in the longer term are discussed below.

3.5.1 Cover crops did not affect maize litter decomposition

Rye and bluegrass cover crops in maize did not affect decomposition rate, litter chemistry, or microbial composition of aboveground maize residue, suggesting that they do not affect the conversion of maize litter to SOC. This finding should mollify grower concerns that cover crops may increase maize residue accumulation and lower subsequent yields in northern climates (Vanhie et al., 2015). However, the result was surprising because growing cover crops have been shown to host unique phyllosphere communities (Bulgarelli et al., 2013). That said, microbial composition changes rapidly after leaf abscission, suggesting that similar microbial communities dominate dead litter irrespective of live-plant assemblages (Tláskal et al., 2016; Voříšková and Baldrian, 2013). Litter type has been shown to drive the composition of the decomposer community (Bray et al., 2012) and decay rates (Moore et al., 2017), but we saw no effects of altered litter quality as a result of cover crop presence. But, it is plausible that contact between maize residue and living cover crops was insufficient for significant transfer of microbes from cover crops to maize residues.

3.5.2 Cover crops increased active C without altering soil microbial composition

Our cover crops did not affect the soil microbial composition after three seasons, unlike other studies observing increases in microbial biomass and shifts in soil microbial composition under cover crops (Finney et al., 2017; Mbuthia et al., 2015; Wortman et al., 2013). Cover crop effects on microbial communities may be dependent on season. For example, Finney et al. (2017) found a greater effect of cover crop on microbial community in fall than spring, but Wortman et al. (2013) found that the early spring plant community had a strong effect on soil microbial composition. In our study, shallow soil sampling under litterbags may not have captured effects of cover crop root exudates on soil microbes (Austin et al., 2017; El et al., 2014) because the primary C inputs for our samples would have come from decomposing maize residue on the surface. Altered litter chemistry may have selected for the litter microbial community over time, since litter quality is a strong driver of bacterial community composition (Cleveland et al., 2014).

The metric PMC represents microbially-available soil C, increases with practices that promote rapid nutrient mineralization, and has been shown to be positively correlated with crop yield (Culman et al., 2013; Hurisso et al., 2016). Increasing PMC under cover crops with no effect on microbial composition and no change in residue decomposition rate indicates that similar microbial communities may be more efficient in converting C inputs into PMC with cover crops (Dijkstra et al., 2015; Frey et al., 2013; Öquist et al., 2017). This could arise from higher cover crop litter-N inputs (as shown by higher POM-N with bluegrass), or cooler temperatures under cover crops, which are known to increase C use efficiency of microbes (Frey et al., 2013; Manzoni, 2017). Either way, more efficient use of C by microbes is likely to increase formation of stable soil C (Cotrufo et al., 2013).

In contrast, POM represents the early stage accrual of litter particulate, and increased with residue inputs in our study, which aligns with previous findings showing a correlation between POM-C and

belowground NPP (Cates et al., 2016; Osborne et al., 2014). However, small quantities of cover crop residue C may have a greater impact where the total residue inputs are lower, as indicated by the increase in total POM with cover crops under silage harvest. Increasing the quantity of reside left in place was more important than total productivity for POM, which was not correlated to any NPP metric. Our positive correlations between NPP and PMC are consistent with the theory that growing plants stimulate microbial activity during their growth (Hendrix et al., 1988), with the potential for accumulation of soil C in the form of microbial necromass (Kallenbach et al., 2015). Our study supports this theory and suggests that over time, increasing NPP will also increase microbially available C. However, more research is needed to determine under what conditions microbes most effectively transfer C inputs not only to PMC, but also to long-term C stocks.

3.5.3 Implications for long-term soil C accrual through cover crops

Evidence shows agroecosystems based on perennial plants are more likely to increase SOC stocks (Tilman et al., 2006) and active C pools (Tiemann and Grandy, 2015). The established roots of perennial grasslands are more fibrous and abundant than annuals, with more exudates and turnover to stimulate microbes throughout the year (Anderson-Teixeira et al., 2013). These traits stimulate a higher degree of rhizosphere microbial activity (Jesus et al., 2016), distribute C throughout the soil profile (Jelinski and Kucharik, 2009), and enhance soil structure (Lehmann et al., 2017; Leifheit et al., 2014; Wilson et al., 2009). Given this paradigm, we would expect that quasi-perennial agroecosystem comprised of both main- and cover-crops might increase soil C as well (Crews and Rumsey, 2017; King and Blesh, 2018).

Our results did not support predictions of increasing SOC stocks with widespread adoption of cover crops (Blanco-Canqui et al., 2015; Poeplau and Don, 2015). It may be because our site–a productive Mollisol–has reached its C-storage capacity under annual agriculture in a northern climate (Sanford et al., 2012; Stewart et al., 2007). In addition, it may be difficult to detect differences in SOC stocks in an

agroecosystem not at equilibrium under a changing climate (Brye et al., 2002; Kucharik et al., 2010). Climate change may be increasing losses of soil C, obscuring any effect of agronomic management, as the growing season has lengthened by 5 to 20 days and precipitation has increased by ~50 mm between 1950 and 2006 in Wisconsin (Jones et al., 2005; Kucharik et al., 2010). Last, cover crops do not provide sufficient habitat for abundant mesofauna, whose composition may increase or decrease nutrient cycling via interactions with microbes and lead to SOC accumulation (Fox et al., 2016; Hawlena et al., 2012; Leslie et al., 2017; Soong et al., 2016; Yang and Gratton, 2014).

Many studies have found equivocal responses of SOC to cover crops (Bich et al., 2014; Kaspar et al., 2006; Snapp and Surapur, 2018; Stetson et al., 2012), indicating that cover crop effects on SOC are likely to vary across the landscape. While it is still unclear whether microbial stimulation or direct belowground biomass inputs are more critical for increasing SOC, annual cover crops only partially mimic these perennial functions, leading to the positive, but limited, increases in active C in this study and others (Blanco-Canqui et al., 2015; Faé et al., 2009; Snapp and Surapur, 2018).

3.6. Conclusions

After 3 years of cover cropping, we found evidence that cover crops increased active soil C pools despite no change in microbial composition or activity. These active C pools were positively correlated to total residue inputs as well as total NPP. However, decomposition of maize residue was not affected by cover crops, suggesting that the potential of cover crops to increase the total soil C is limited to belowground processes. While belowground C is preferentially assimilated by microbes and stabilized via organo-mineral associations (Hicks Pries et al., 2017; Rasse et al., 2005), in agricultural systems a large portion of C inputs come from aboveground. Future research should evaluate the mechanisms by which aboveground litter can be incorporated into soil C and explore agricultural innovations that maximize belowground C, such as incorporation of perennials into rotations and breeding for increased root

production in annual crops. The positive but small effects of cover crops on soil C suggest that their promotion should focus on water quality and soil protection benefits rather than their potential to help store C in soils.

3.7. Acknowledgments

We are grateful to L. Gary Oates, Gregg Sanford, Alex Butz, Mark Walsh, Jimmy Sustachek, Casey Menick, Sarah Lee and Kelsey Johnson for field and laboratory assistance. Funding was provided by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DEFC0207ER64494).

3.8. References

Al-Kaisi, M.M., Kwaw-Mensah, D., Ci, E., 2017. Effect of nitrogen fertilizer application on corn residue decomposition in Iowa. Agronomy Journal 109, 2415. doi:10.2134/agronj2016.11.0633

Anderson-Teixeira, K.J., Masters, M.D., Black, C.K., Zeri, M., Hussain, M.Z., Bernacchi, C.J., DeLucia, E.H., 2013. Altered belowground carbon cycling following land-use change to perennial bioenergy crops. Ecosystems 16, 508–520. doi:10.1007/s10021-012-9628-x

Austin, E.E., Wickings, K., McDaniel, M.D., Robertson, G.P., Grandy, A.S., 2017. Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. GCB Bioenergy 9, 1252–1263. doi:10.1111/gcbb.12428

Baker, J.M., Griffis, T.J., 2005. Examining strategies to improve the carbon balance of corn/soybean agriculture using eddy covariance and mass balance techniques. Agricultural and Forest Meteorology 128, 163–177. doi:10.1016/j.agrformet.2004.11.005

Bich, A.D., Reese, C.L., Kennedy, A.C., Clay, D.E., Clay, S.A., 2014. Corn yield is not reduced by mid-season establishment of cover crops in northern great plains environments. Crop Management 13. doi:10.2134/CM-2014-0009-RS

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, 2449–2474. doi:10.2134/agronj15.0086

Blubaugh, C.K., Hagler, J.R., Machtley, S.A., Kaplan, I., 2016. Cover crops increase foraging activity of omnivorous predators in seed patches and facilitate weed biological control. Agriculture, Ecosystems & Environment 231, 264–270. doi:10.1016/J.AGEE.2016.06.045

Bray, S.R., Kitajima, K., Mack, M.C., 2012. Temporal dynamics of microbial communities on decomposing leaf litter of 10 plant species in relation to decomposition rate. Soil Biology and Biochemistry 49, 30–37. doi:10.1016/j.soilbio.2012.02.009

Brye, K.R., Gower, S.T., Norman, J.M., Bundy, L.G., 2002. Carbon budgets for a prairie and agroecosystem: effects of land use and interannual variability. Ecological Applications 12, 962–979. doi:10.1890/1051-0761(2002)012[0962:CBFAPA]2.0.CO;2

Bulgarelli, D., Schlaeppi, K., Spaepen, S., van Themaat, E.V.L., Schulze-Lefert, P., 2013. Structure and Functions of the Bacterial Microbiota of Plants. Annual Review of Plant Biology 64, 807–838. doi:10.1146/annurev-arplant-050312-120106

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial Enzyme Shifts Explain Litter Decay Responses to Simulated Nitrogen Deposition. Ecology 81, 2359. doi:10.2307/177459

Cates, A.M., Jackson, R.D., 2018. Cover crop effects on net ecosystem carbon balance in grain and silage maize. Agronomy Journal 0, 1–29. doi:10.1128/JCM.00049-14

Cates, A.M., Ruark, M.D., Hedtcke, J.L., Posner, J.L., 2016. Long-term tillage, rotation and perennialization effects on particulate and aggregate soil organic matter. Soil and Tillage Research 155, 371–380. doi:10.1016/j.still.2015.09.008

Chen, Y., Ma, S., Liu, J., Cheng, G., Lu, X., 2018. Soil C and N dynamics and their non-additive responses to litter mixture under different moisture conditions from an alpine steppe soil, Northern Tibet. Soil Biology and Biochemistry 125, 231–238. doi:10.1016/J.SOILBIO.2018.07.016

Cheng, W., 2009. Rhizosphere priming effect: Its functional relationships with microbial turnover, evapotranspiration, and C–N budgets. Soil Biology and Biochemistry 41, 1795–1801. doi:10.1016/j.soilbio.2008.04.018

Cleveland, C.C., Reed, S.C., Keller, A.B., Nemergut, D.R., O'Neill, S.P., Ostertag, R., Vitousek, P.M., 2014. Litter quality versus soil microbial community controls over decomposition: a quantitative analysis. Oecologia 174, 283–94. doi:10.1007/s00442-013-2758-9

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

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988–95. doi:10.1111/gcb.12113

Crews, T., Rumsey, B., 2017. What Agriculture Can Learn from Native Ecosystems in Building Soil Organic Matter: A Review. Sustainability 9, 578. doi:10.3390/su9040578

Culman, S.W., Snapp, S.S., Green, J.M., Gentry, L.E., 2013. Short- and long-term labile soil carbon and nitrogen dynamics reflect management and predict corn agronomic performance. Agronomy Journal 105, 493–502. doi:10.2134/agronj2012.0382

Dijkstra, F.A., Bader, N.E., Johnson, D.W., Cheng, W., 2009. Does accelerated soil organic matter decomposition in the presence of plants increase plant N availability? Soil Biology and Biochemistry 41, 1080–1087. doi:10.1016/j.soilbio.2009.02.013

Dijkstra, F.A., Morgan, J.A., Blumenthal, D., Follett, R.F., 2010. Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. Soil Biology and Biochemistry 42, 1073–1082. doi:10.1016/j.soilbio.2010.02.026

Dijkstra, P., Salpas, E., Fairbanks, D., Miller, E.B., Hagerty, S.B., van Groenigen, K.J., Hungate, B.A., Marks,

J.C., Koch, G.W., Schwartz, E., 2015. High carbon use efficiency in soil microbial communities is related to balanced growth, not storage compound synthesis. Soil Biology and Biochemistry 89, 35–43. doi:10.1016/j.soilbio.2015.06.021

El, F., Haichar, Z., Santaella, C., Heulin, T., Achouak, W., 2014. Root exudates mediated interactions belowground. doi:10.1016/j.soilbio.2014.06.017

Faé, G.S., Mark Sulc, R., Barker, D.J., Dick, R.P., Eastridge, M.L., Lorenz, N., 2009. Integrating winter annual forages into a no-till corn silage system. Agronomy Journal 101, 1286–1296. doi:10.2134/agronj2009.0144

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, 361–373. doi:10.2489/jswc.72.4.361

Flerchinger, G.N., Sauer, T.J., Aiken, R.A., 2003. Effects of crop residue cover and architecture on heat and water transfer at the soil surface. Geoderma 116, 217–233. doi:10.1016/S0016-7061(03)00102-2

Fox, A.F., Kim, T.N., Bahlai, C.A., Woltz, J.M., Gratton, C., Landis, D.A., 2016. Cover crops have neutral effects on predator communities and biological control services in annual cellulosic bioenergy cropping systems. Agriculture, Ecosystems and Environment 232, 101–109. doi:10.1016/j.agee.2016.07.003

Franzluebbers, A.J., Haney, R.L., Honeycutt, C.W., Schomberg, H.H., Hons, F.M., 2000. Flush of Carbon Dioxide Following Rewetting of Dried Soil Relates to Active Organic Pools. Soil Science Society of America Journal 64, 613. doi:10.2136/sssaj2000.642613x

Frey, S.D., Lee, J., Melillo, J.M., Six, J., 2013. The temperature response of soil microbial efficiency and its

feedback to climate. Nature Climate Change 3, 395–398. doi:10.1038/nclimate1796

Gebremedhin, M.T., Loescher, H.W., Tsegaye, T.D., 2012. Carbon balance of no-till soybean with winter wheat cover crop in the Southeastern United States. Agronomy Journal 104, 1321–1335. doi:10.2134/agronj2012.0072

Grandy, A.S., Strickland, M.S., Lauber, C.L., Bradford, M.A., Fierer, N., 2009. The influence of microbial communities, management, and soil texture on soil organic matter chemistry. Geoderma 150, 278–286. doi:10.1016/j.geoderma.2009.02.007

Hawlena, D., Strickland, M.S., Bradford, M.A., Schmitz, O.J., 2012. Fear of predation slows plant-litter decomposition. Science (New York, N.Y.) 336, 1434–8. doi:10.1126/science.1220097

Hendrix, P.F., HAN, C.-R., Groffman, P.M., 1988. Soil respiration in conventional and no-tillage agroecosystems under different winter cover crop rotations. Soil and Tillage Research 12, 135–148. doi:10.1016/0167-1987(88)90037-2

Hicks Pries, C.E., Bird, J.A., Castanha, C., Hatton, P.-J., Torn, M.S., 2017. Long term decomposition: the influence of litter type and soil horizon on retention of plant carbon and nitrogen in soils. Biogeochemistry 134, 5–16. doi:10.1007/s10533-017-0345-6

Hurisso, T.T., Culman, S.W., Horwath, W.R., Wade, J., Cass, D., Beniston, J.W., Bowles, T.M., Grandy, A.S., Franzluebbers, A.J., Schipanski, M.E., Lucas, S.T., Ugarte, C.M., 2016. Comparison of permanganate-oxidizable carbon and mineralizable carbon for assessment of organic matter stabilization and mineralization. Soil Science Society of America Journal 80, 1352. doi:10.2136/sssaj2016.04.0106

Jelinski, N.A., Kucharik, C.J., 2009. Land-use effects on soil carbon and nitrogen on a US midwestern floodplain. Soil Science Society of America Journal 73, 217–225.

Jesus, E. da C., Liang, C., Quensen, J.F., Susilawati, E., Jackson, R.D., Balser, T.C., Tiedje, J.M., 2016.

Influence of corn, switchgrass, and prairie cropping systems on soil microbial communities in the upper Midwest of the United States. GCB Bioenergy 8, 481–494. doi:10.1111/gcbb.12289

Jones, C., McConnell, C., Coleman, K., Cox, P., Falloon, P., Jenkinson, D., Powlson, D., 2005. Global climate change and soil carbon stocks; predictions from two contrasting models for the turnover of organic carbon in soil. Global Change Biology 11, 154–166. doi:10.1111/j.1365-2486.2004.00885.x

Kallenbach, C.M., Grandy, A.S., Frey, S.D., Diefendorf, A.F., 2015. Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biology and Biochemistry 91, 279–290. doi:10.1016/j.soilbio.2015.09.005

Kallenbach, C.M., Grandy, S.A., 2015. Land use legacies regulate decomposition dynamics following bioenergy crop conversion. GCB Bioenergy 7, 1232–1244. doi:10.1111/gcbb.12218

Kaspar, T.C., Parkin, T.B., Jaynes, D.B., Cambardella, C.A., Meek, D.W., Jung, Y.S., 2006. Examining changes in soil organic carbon with oat and rye cover crops using terrain covariates. Soil Science Society of America Journal 70, 1168. doi:10.2136/sssaj2005.0095

Keiblinger, K.M., Schneider, T., Roschitzki, B., Schmid, E., Eberl, L., Hämmerle, I., Leitner, S., Richter, A., Wanek, W., Riedel, K., Zechmeister-Boltenstern, S., 2012. Effects of stoichiometry and temperature perturbations on beech leaf litter decomposition, enzyme activities and protein expression. Biogeosciences 9, 4537–4551. doi:10.5194/bg-9-4537-2012

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

Kucharik, C.J., Serbin, S.P., Vavrus, S., Hopkins, E.J., Motew, M.M., 2010. Patterns of climate change across Wisconsin from 1950 to 2006. Physical Geography 31, 1–28. doi:10.2747/0272-3646.31.1.1

Ladoni, M., Basir, A., Robertson, P.G., Kravchenko, A.N., 2016. Scaling-up: cover crops differentially

influence soil carbon in agricultural fields with diverse topography. Agriculture, Ecosystems & Environment 225, 93–103. doi:10.1016/j.agee.2016.03.021

Lehmann, A., Leifheit, E.F., Rillig, M.C., 2017. Chapter 14: Mycorrhizas and Soil Aggregation, in: Johnson, N.C., Gehring, C.A., Jansa, J. (Eds.), Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage. Elsevier Inc., p. 509. doi:10.1016/B978-0-12-804312-7.00014-0

Leifheit, E.F., Veresoglou, S.D., Lehmann, A., Morris, E.K., Rillig, M.C., 2014. Multiple factors influence the role of arbuscular mycorrhizal fungi in soil aggregation–a meta-analysis. Plant and Soil 374, 523–537. doi:10.1007/s11104-013-1899-2

Leslie, A.W., Wang, K.-H., Meyer, S.L.F., Marahatta, S., Hooks, C.R.R., 2017. Influence of cover crops on arthropods, free-living nematodes, and yield in a succeeding no-till soybean crop. Applied Soil Ecology 117–118, 21–31. doi:10.1016/J.APSOIL.2017.04.003

Manzoni, S., 2017. Flexible carbon-use efficiency across litter types and during decomposition partly compensates nutrient imbalances—results from analytical stoichiometric models. Frontiers in Microbiology 8, 661. doi:10.3389/fmicb.2017.00661

Mazzilli, S.R., Kemanian, A.R., Ernst, O.R., Jackson, R.B., Piñeiro, G., 2015. Greater humification of belowground than aboveground biomass carbon into particulate soil organic matter in no-till corn and soybean crops. Soil Biology and Biochemistry 85, 22–30. doi:10.1016/J.SOILBIO.2015.02.014

Mbuthia, L.W., Acosta-Martínez, V., DeBruyn, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N., DeBryun, J., Schaeffer, S., Tyler, D., Odoi, E., Mpheshea, M., Walker, F., Eash, N., 2015. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. Soil Biology and Biochemistry 89, 24–34. doi:10.1016/j.soilbio.2015.06.016

McDaniel, M.D., Grandy, A.S., Tiemann, L.K., Weintraub, M.N., 2014. Crop rotation complexity regulates the decomposition of high and low quality residues. Soil Biology and Biochemistry 78, 243–254. doi:10.1016/j.soilbio.2014.07.027

Moore, T.R., Trofymow, J.A., Prescott, C.E., Titus, B.D., Group, the C.W., 2017. Can short-term litter-bag measurements predict long-term decomposition in northern forests? Plant and Soil 416, 419–426. doi:10.1007/s11104-017-3228-7

Muñoz, J.D., Steibel, J.P., Snapp, S., Kravchenko, A.N., 2014. Cover crop effect on corn growth and yield as influenced by topography. Agriculture, Ecosystems and Environment 189, 229–239. doi:10.1016/j.agee.2014.03.045

Öquist, M.G., Erhagen, B., Haei, M., Sparrman, T., Ilstedt, U., Schleucher, J., Nilsson, M.B., 2017. The effect of temperature and substrate quality on the carbon use efficiency of saprotrophic decomposition. Plant and Soil 414, 113–125. doi:10.1007/s11104-016-3104-x

Osborne, S.L., Johnson, J.M.F., Jin, V.L., Hammerbeck, A.L., Varvel, G.E., Schumacher, T.E., 2014. The impact of corn residue removal on soil aggregates and particulate organic matter. BioEnergy Research 7, 559–567. doi:10.1007/s12155-014-9413-0

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

R Core Team, 2016. R: a Language and Environment for Statistical Computing.

Rasse, D.P., Rumpel, C., Dignac, M.F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant and Soil 269, 341–356. doi:10.1007/s11104-004-0907-y

Saiya-Cork, K.., Sinsabaugh, R.., Zak, D.., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology and Biochemistry 34,

1309–1315. doi:10.1016/S0038-0717(02)00074-3

Sanford, G.R., Posner, J.L., Jackson, R.D., Kucharik, C.J., Hedtcke, J.L., Lin, T.L., 2012. Soil carbon lost from Mollisols of the North Central U.S.A. with 20 years of agricultural best management practices. Agriculture, Ecosystems and Environment 162, 68–76. doi:10.1016/j.agee.2012.08.011

Snapp, S., Surapur, S., 2018. Rye cover crop retains nitrogen and doesn't reduce corn yields. Soil and Tillage Research 180, 107–115. doi:10.1016/J.STILL.2018.02.018

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

Steinweg, J.M., Dukes, J.S., Wallenstein, M.D., 2012. Modeling the effects of temperature and moisture on soil enzyme activity: Linking laboratory assays to continuous field data. Soil Biology and Biochemistry 55, 85–92. doi:10.1016/j.soilbio.2012.06.015

Stetson, S.J., Osborne, S.L., Schumacher(Retired), T.E., Eynard, A., Chilom, G., Rice, J., Nichols, K.A., Pikul, J.L., 2012. Corn Residue Removal Impact on Topsoil Organic Carbon in a Corn–Soybean Rotation. Soil Science Society of America Journal 76, 1399. doi:10.2136/sssaj2011.0420

Stewart, C.E., Paustian, K., Conant, R.T., Plante, A.F., Six, J., 2007. Soil carbon saturation: concept, evidence and evaluation. Biogeochemistry 86, 19–31. doi:10.1007/s10533-007-9140-0

Tiemann, L.K., Grandy, A.S., 2015. Mechanisms of soil carbon accrual and storage in bioenergy cropping systems. GCB Bioenergy 7, 161–174. doi:10.1111/gcbb.12126

Tilman, D., Hill, J., Lehman, C., 2006. Carbon-negative biofuels from low-input high-diversity grassland biomass. Science 314, 1598–1600. doi:10.1126/science.1133306

Tláskal, V., Voříšková, J., Baldrian, P., 2016. Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. FEMS Microbiology Ecology 92, fiw177. doi:10.1093/femsec/fiw177

Vanhie, M., Deen, W., Lauzon, J.D., Hooker, D.C., 2015. Effect of increasing levels of maize (Zea mays L.) residue on no-till soybean (Glycine max Merr.) in Northern production regions: A review. Soil and Tillage Research 150, 201–210. doi:10.1016/J.STILL.2015.01.011

Varela, M.F., Scianca, C.M., Taboada, M.A., Rubio, G., 2014. Cover crop effects on soybean residue decomposition and P release in no-tillage systems of Argentina. Soil and Tillage Research 143, 59–66. doi:10.1016/j.still.2014.05.005

Voříšková, J., Baldrian, P., 2013. Fungal community on decomposing leaf litter undergoes rapid successional changes. The ISME Journal 7, 477–86. doi:10.1038/ismej.2012.116

Wickings, K., Grandy, A.S., 2013. Management intensity interacts with litter chemistry and climate to drive temporal patterns in arthropod communities during decomposition. Pedobiologia 56, 105–112. doi:10.1016/j.pedobi.2013.01.001

Wilson, G.W.T., Rice, C.W., Rillig, M.C., Springer, A., Hartnett, D.C., 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: Results from long-term field experiments. Ecology Letters 12, 452–461. doi:10.1111/j.1461-0248.2009.01303.x

Wortman, S.E., Drijber, R.A., Francis, C.A., Lindquist, J.L., 2013. Arable weeds, cover crops, and tillage drive soil microbial community composition in organic cropping systems. Applied Soil Ecology 72, 232–241. doi:10.1016/j.apsoil.2013.07.014

Yang, L.H., Gratton, C., 2014. Insects as drivers of ecosystem processes. Current Opinion in Insect

Science 2, 26–32. doi:10.1016/j.cois.2014.06.004

Zhang, W., Yuan, S., Hu, N., Lou, Y., Wang, S., 2015. Predicting soil fauna effect on plant litter decomposition by using boosted regression trees. Soil Biology and Biochemistry 82, 81–86. doi:10.1016/j.soilbio.2014.12.016

Zhu, B., Gutknecht, J.L.M., Herman, D.J., Keck, D.C., Firestone, M.K., Cheng, W., 2014. Rhizosphere priming effects on soil carbon and nitrogen mineralization. Soil Biology and Biochemistry 76, 183–192. doi:10.1016/j.soilbio.2014.04.033

3.9. Tables and Figures

Table 1. Decomposition parameters after 18 months of decomposition. Decomposition rate (k) of total litter, litter C, or litter N by day calculated from exponential decay models. Mass is the ash-free mass remaining (%) and enzyme efficiency represents the sum of enzyme activity per litter-C mass lost. Standard errors are given in parentheses. No statistical differences among cover crop treatments were found.

Cover crop	Litter-k ($g\ day^{-1}$)	C-k ($g\ day^{-1}$)	N-k ($g\ day^{-1}$)	Mass (%)	Enzyme efficiency ($\mu mol\ EEA\ g\ C\ lost^{-1}$)
Bluegrass	-0.0022	-0.0057	-0.0025	28 (0.02)	5.86 (0.81)
Rye	-0.0021	-0.0058	-0.0026	32 (0.04)	8.59 (1.10)
No cover	-0.0019	-0.0051	-0.0023	32 (0.04)	8.65 (1.36)

Table 2. Mean values (SE) of soil organic C, potentially mineralizable C, and particulate organic matter (SOC, PMC and POM) after 3 years of cover cropping in continuous maize harvested for grain or silage. Lowercase letters after means represent differences among cover crop treatments within harvest treatments and soil depth.

Depth		SOC	PMC		POM		POM-C		POM-N	
Harvest	Cover crop	(Mg C ha^{-1})	(μg CO_2-C g $soil^{-1}$)		(g kg $soil^{-1}$)		(g kg $soil^{-1}$)		(g kg $soil^{-1}$)	
0-10 cm										
Grain	Bluegrass	28.8 (1.4)	148.5 (2.8)	ab	67.9 (3.9)	a	4.99 (0.32)	a	0.258 (0.020)	a
	No Cover	29.3 (1.6)	139.5 (1.9)	b	65.7 (4.2)	ab	4.27 (0.32)	ab	0.218 (0.017)	ab
	Rye	30.5 (1.8)	150.3 (3.3)	a	57.7 (2.3)	b	4.03 (0.46)	b	0.188 (0.019)	b
Silage	Bluegrass	28.5 (0.9)	134.2 (4.2)	b	75.7 (5.9)	a	3.78 (0.28)		0.182 (0.018)	
	No Cover	27.0 (1.3)	109.9 (3.2)	c	62.0 (2.6)	b	3.35 (0.28)		0.175 (0.017)	
	Rye	27.8 (1.7)	147.7 (5.2)	a	71.4 (5.6)	ab	3.60 (0.27)		0.186 (0.019)	
10-25 cm										
Grain	Bluegrass	38.2 (2.5)	76.8 (2.1)	b	52.5 (4.0)		1.69 (0.22)		0.090 (0.01)	
	No Cover	38.8 (2.5)	77.4 (1.4)	b	55.6 (3.9)		2.02 (0.25)		0.105 (0.01)	
	Rye	40.9 (1.9)	84.5 (2.9)	a	48.1 (3.1)		1.33 (0.15)		0.079 (0.007)	
Silage	Bluegrass	37.3 (2.4)	82.4 (3.8)	a	52.8 (1.6)		1.26 (0.09)		0.074 (0.007)	
	No Cover	37.4 (1.9)	72.0 (2.4)	b	47.3 (1.8)		1.37 (0.10)		0.077 (0.005)	
	Rye	38.4 (2.2)	85.1 (2.6)	a	51.1 (4.6)		1.22 (0.11)		0.075 (0.006)	

Figure 1. Non-metric multidimensional scaling ordination of maize litter (a) molecular C composition as assessed by py-GC/MS and (b) microbial composition as assessed by metagenomic profiling.

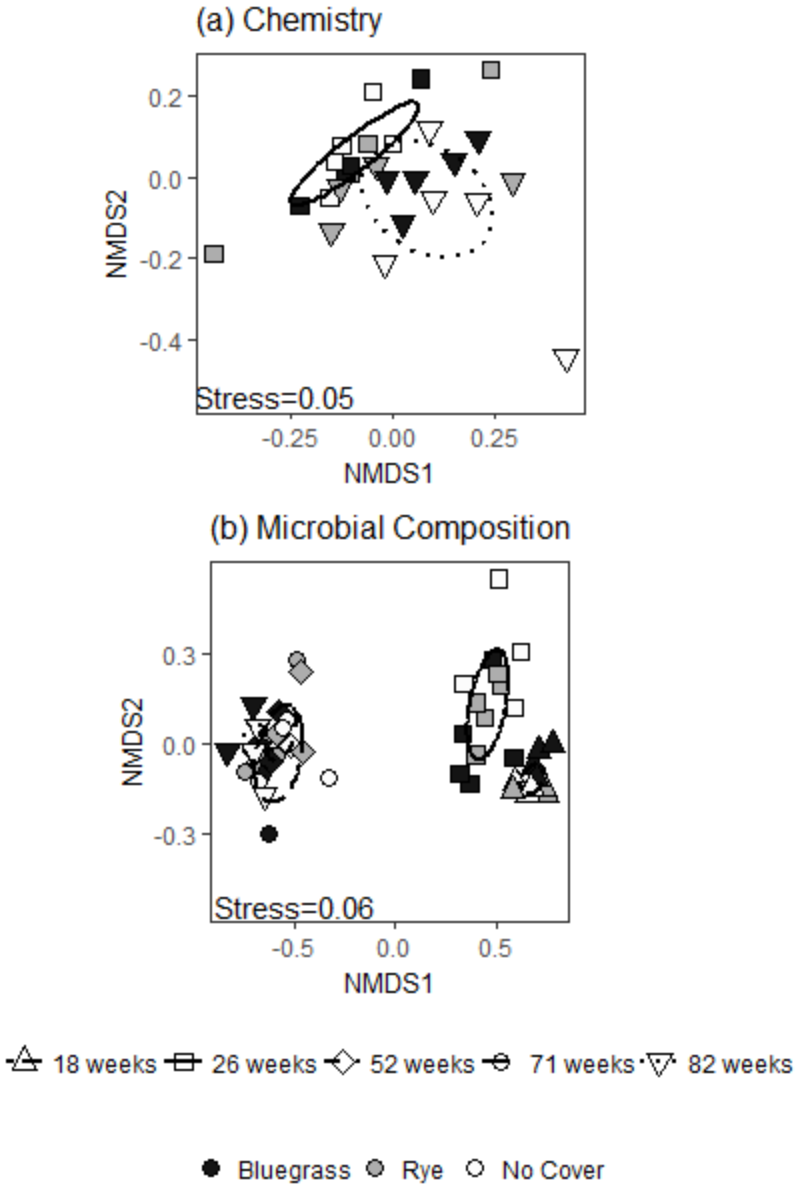


Figure 2. Changes in (a) soil C and (b) soil N and non-metric multidimensional scaling ordination of (c) soil molecular C composition as assessed by py-GC/MS and (d) soil microbial composition as assessed by metagenomic profiling. Soil was collected immediately below maize-filled litterbags after 18, 26, 34, 52, 71 and 82 weeks of decomposition.

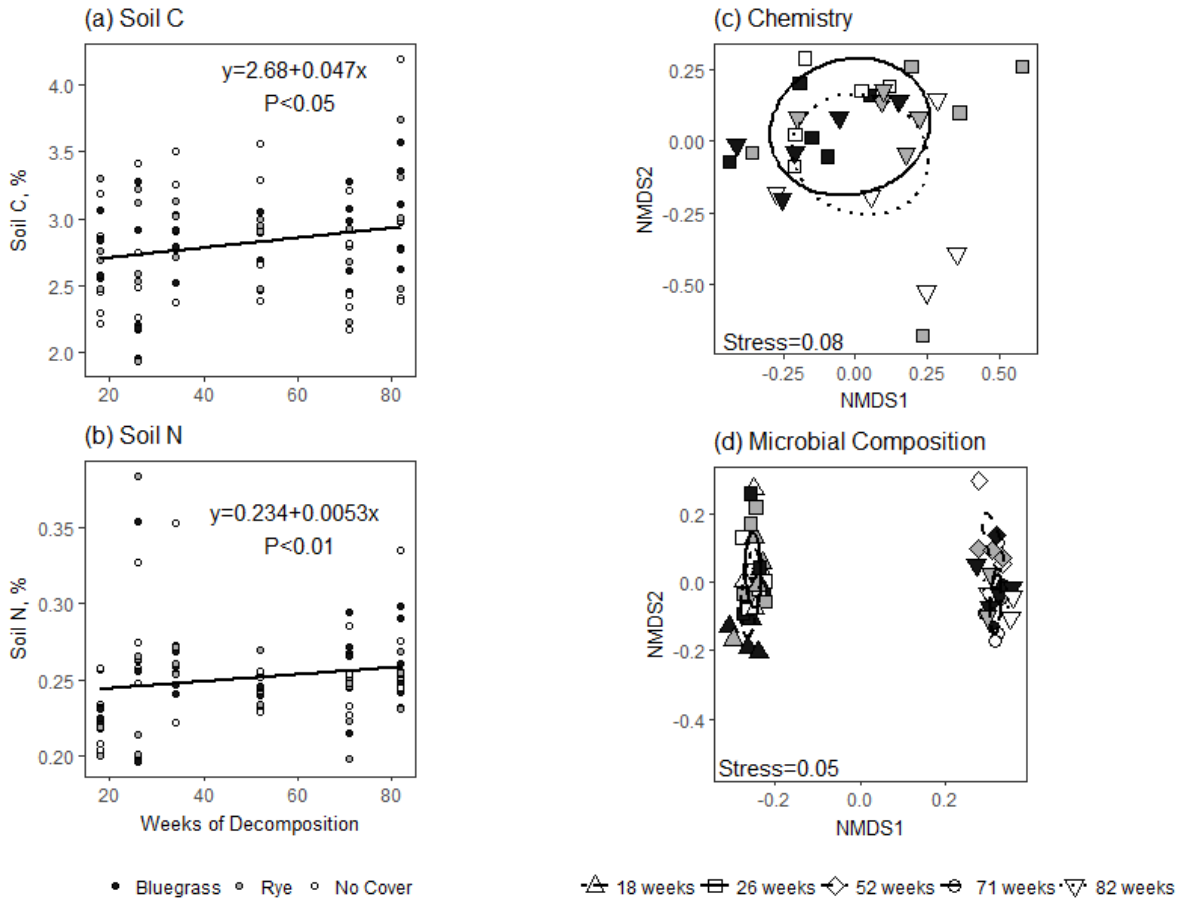
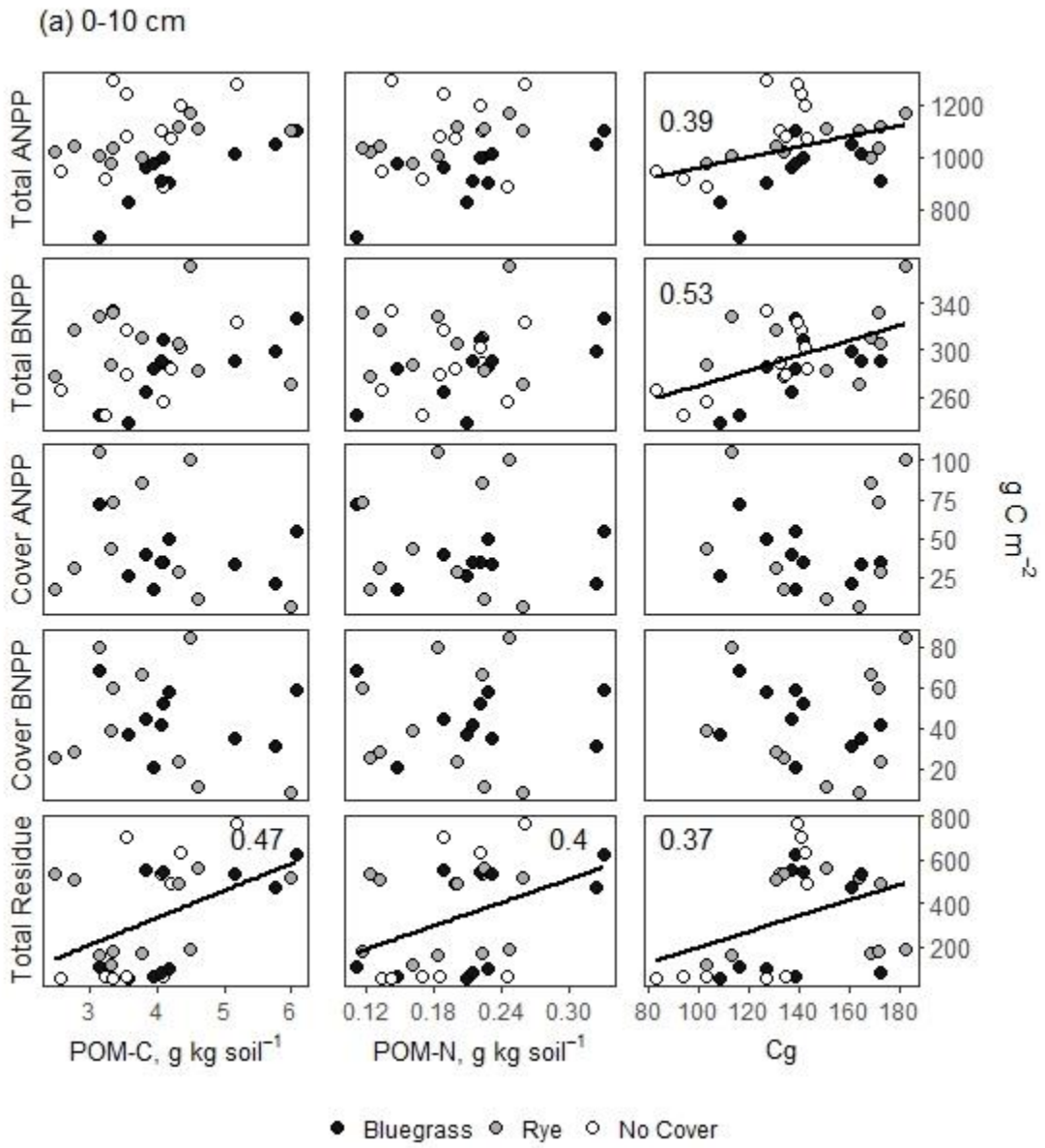
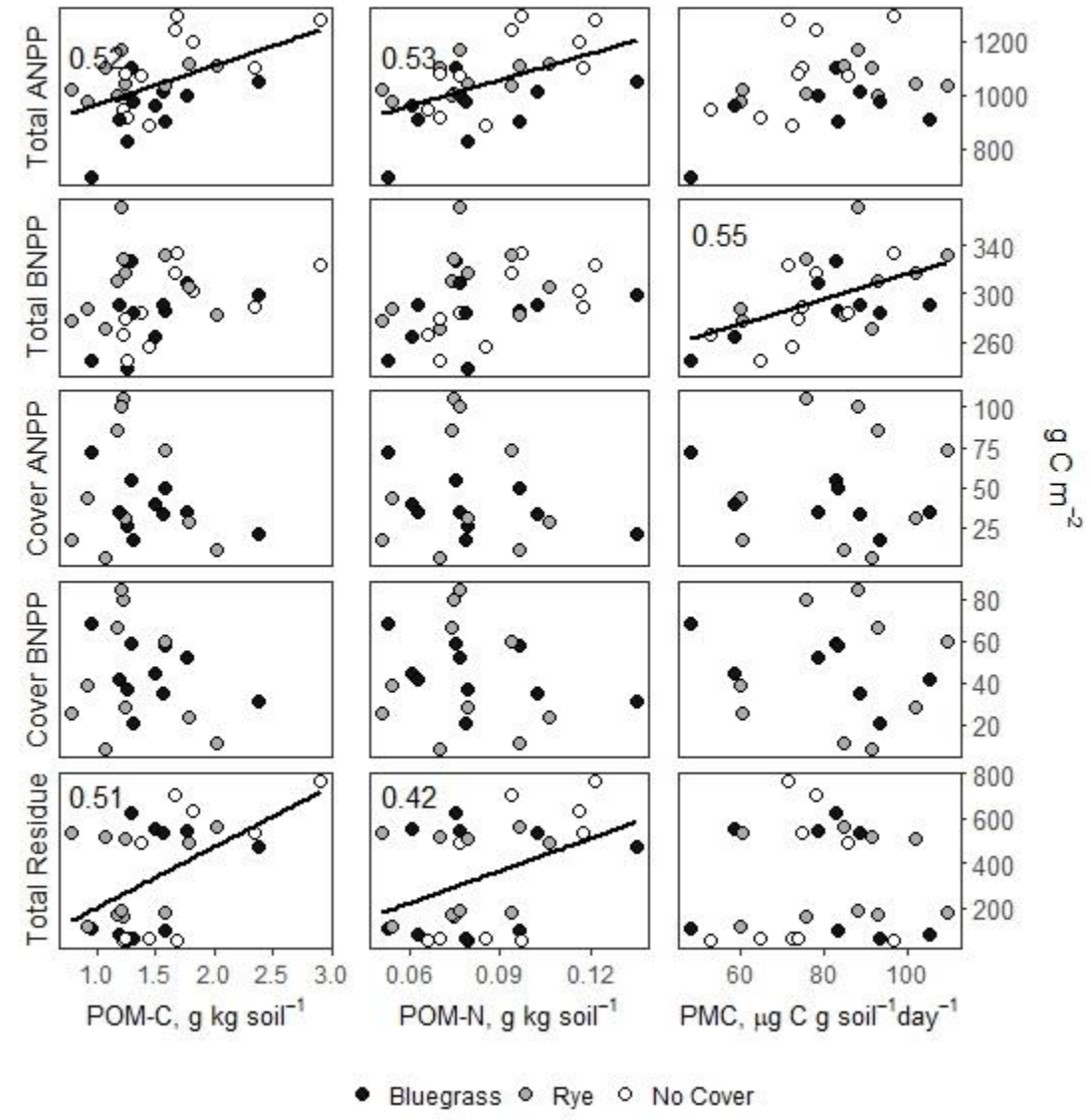


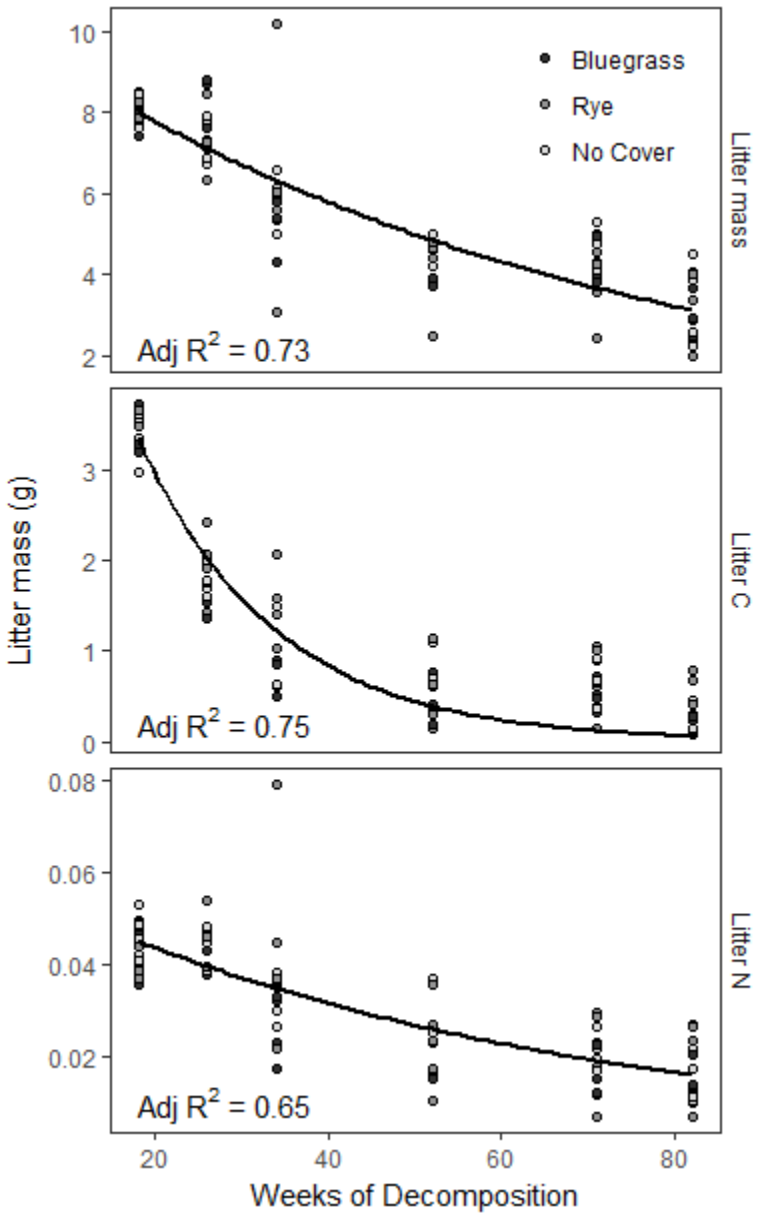
Figure 3. Correlations between mean C in above- and below-ground net primary productivity (ANPP and BNPP) of maize/cover crop systems and active C fractions at a) 0 to 10 cm and b) 10 to 25 cm soil depth. POM is particulate organic matter, and PMC is potentially mineralizable C. Total ANPP and BNPP refer to maize and cover crop biomass C, while Cover ANPP and BNPP refer only to cover crop biomass C. Total residue refers to the sum of all unharvested material, maize and cover crop. R-values are displayed where correlations were significant (P<0.05).



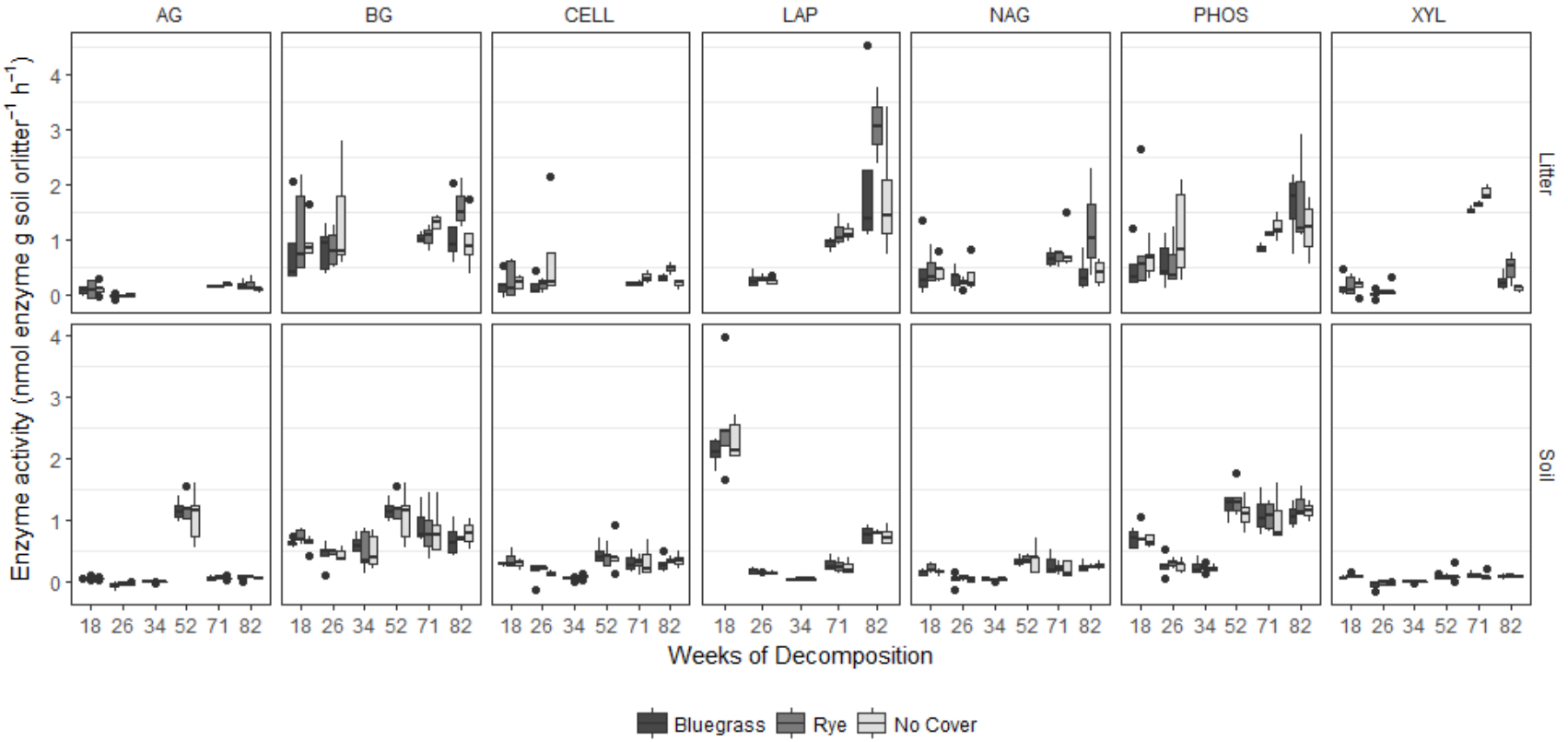
(b) 10-25 cm


Total ANPP
Total BNPP
Cover ANPP
Cover BNPP
Total Residue
0.52
0.53
0.55
0.51
0.42
1200
1000
800
340
300
260
100
75
50
25
80
60
40
20
800
600
400
200
g C m^{-2}
1.0 1.5 2.0 2.5 3.0
POM-C, g kg soil^{-1}
0.06 0.09 0.12
POM-N, g kg soil^{-1}
60 80 100
PMC, μg C g soil^{-1}day^{-1}
Bluegrass
Rye
No Cover

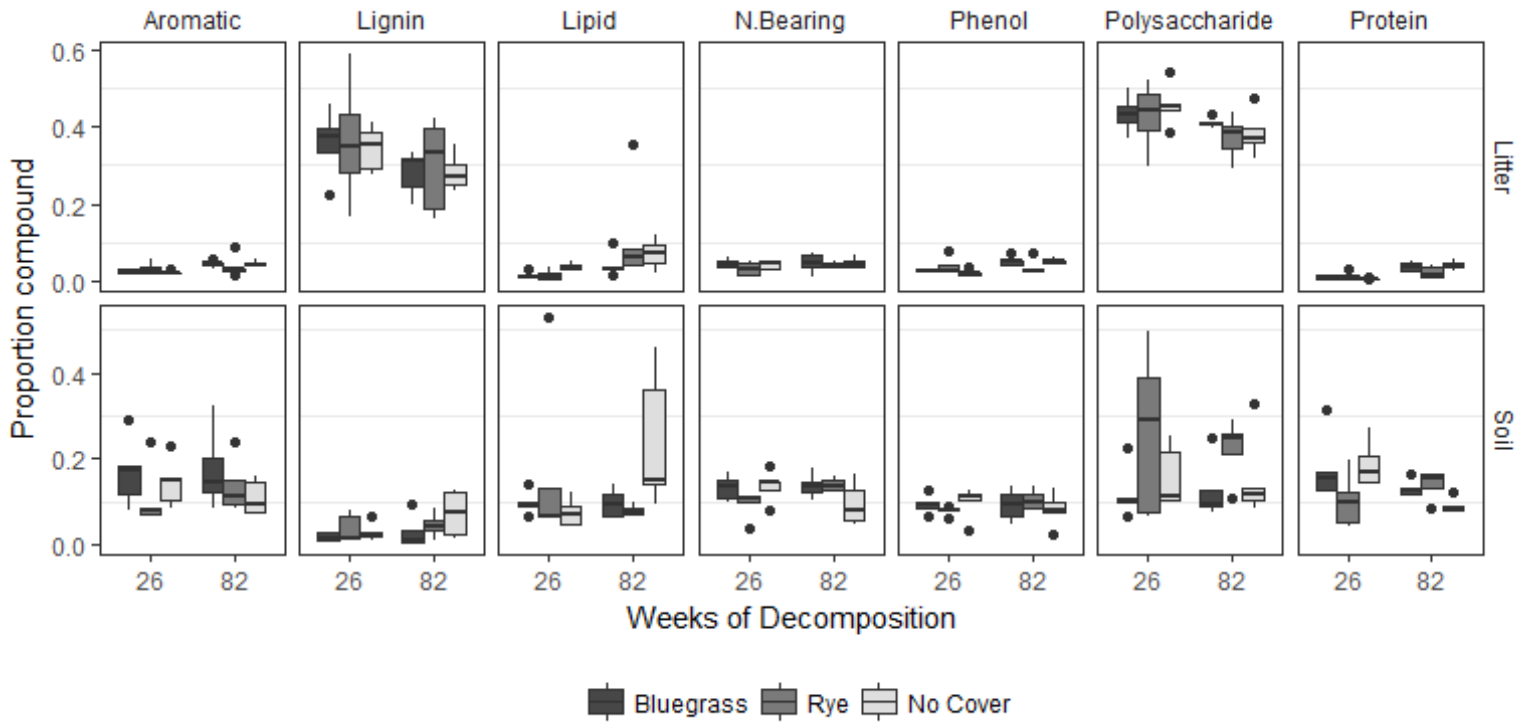
Supplemental Figure 1. Mass and C and N content of maize litter. A single exponential decay model is shown as parameters did not differ among treatments. All models were significant at $P<0.0001$. Litterbags were collected after 18, 26, 34, 52, 71 and 82 weeks of decomposition.



Supplemental Figure 2. Extracellular enzyme activity in maize litter (after 26 and 82 weeks of decomposition in litterbags) and adjacent 0 to 5 cm soil. Enzymes were α-glucosidase, β-glucosidase, N-acetyl-β-glucosidase, β-D-cellobiosidase,, L-Leucine-7-amidomethylcoumarin, phosphatase, and β-xylosidase (AG, BG, CELL, LAP, PHOS, NAG, and XYL).



Supplemental Figure 3. Chemical characterization of maize litter (after 26 and 82 weeks of decomposition in litterbags) and adjacent 0 to 5 cm soil. Compound classes are expressed as the proportion of peak area attributed to that class out of the total peak area in a given sample, as determined by py-GC/MS.



Supplemental Table 1. P-values from 2-way ANOVA comparing the effects of date (26 or 82 weeks of decomposition at sampling), cover crop treatment (rye, bluegrass, or control) and date x cover on proportion of compound classes in maize litter and 0 to 5 cm adjacent soil samples. Compound classes expressed as the proportion of peak area attributed to that class out of the total peak area in a given sample as determined by py-GC/MS.

Source	Aromatic	Lignin	Lipid	Polysaccharide	N-Bearing	Protein	Phenol	Unknown Origin
Litter								
Date	**<0.01**	0.09	**<0.05**	**<0.05**	0.45	**<0.0001**	**<0.05**	0.92
Cover crop	0.89	0.59	0.13	0.86	0.54	0.26	0.63	0.28
Date x Cover	0.68	0.82	0.49	0.41	0.69	**<0.05**	0.19	0.78
Soil								
Date	0.99	0.06	0.49	0.72	0.91	**<0.05**	0.86	0.90
Cover crop	0.23	0.18	0.46	**<0.05**	0.32	0.23	0.98	0.11
Date x Cover	0.59	0.63	**<0.05**	0.84	0.05	**<0.05**	0.32	0.82

Supplemental Table 2. P-values from 2-way ANOVA on extracellular enzyme activity in maize litter and 0 to 5 cm adjacent soil samples. Enzymes measured were α-glucosidase, β-glucosidase, N-acetyl-β-glucosidase, β-D-cellobiosidase, L-Leucine-7-amidomethylcoumarin, phosphatase, and β-xylosidase (AG, BG, CELL, LAP, PHOS, NAG, and XYL), as well as the sum of all enzyme activity, in nmol g^{-1} litter or soil h^{-1}.

Source	AG	BG	CELL	PHOS	NAG	XYL	LAP	Sum
Litter								
Date	**<0.01**	0.22	0.79	**<0.001**	**<0.05**	**<0.001**	**<0.01**	**<0.001**
Cover crop	0.78	0.43	0.32	0.64	0.54	0.67	0.17	0.24
Date x Cover	0.68	0.64	0.41	0.57	0.13	0.80	0.13	**0.05**
Soil								
Date	**0.06**	**<0.01**	**<0.05**	**<0.001**	**<0.001**	**<0.001**	**<0.01**	0.99
Cover crop	0.88	0.83	0.89	0.99	0.69	0.83	0.80	0.84
Date x Cover	0.95	0.93	0.76	0.82	0.94	0.84	0.79	0.94

Chapter 4: Separate drivers for microbial C mineralization and physical protection of C

4.1 Abstract

While we know about the effects of temperature and moisture on soil microbial activity, and that microbial by-products are a critical source of soil C, we are missing a link in our understanding of how physical protection of soil C may be affected by temperature and moisture. We performed a 6-month incubation of soil and plant litter under varying temperature and moisture. Using ^{13}C isotopic differences, we traced plant litter C into various aggregate fractions after 30, 50, and 60% of plant C had been respired. In addition, we evaluated microbial biomass C, enzyme activity, and organic C chemistry in soil aggregates. While warmer temperatures increased C mineralization rate and decreased microbial biomass, aggregation was enhanced under drier conditions irrespective of temperature. Complex C compounds were relatively more abundant under moister conditions, pointing to a greater role for simple compounds in stabilizing macro- and micro-aggregates. Microbial composition shifted over time and with temperature and moisture, but no particular microbial community was associated specifically with the drier conditions that promoted aggregation. These results indicate that physical protection of C in aggregates is more vulnerable to changes in moisture regime than temperature. Predictions of how management and climate will affect soil C storage should incorporate separate responses to temperature and moisture for aggregate and microbial C pools.

4.2 Introduction

Recent evidence suggests that soil organic matter (SOM) is formed both early in litter decomposition, during leaching of non-structural compounds, and late in decomposition, when litter is fragmented and incorporated physically into the soil (Cotrufo et al., 2015). Litter decomposition pathways may be altered by the litter quality (e.g., lignin:N ratio) or by decomposer communities, resulting in different end products (Lavallee et al., 2018; Wickings et al., 2012). Litter decomposition rate is commonly

measured as mass loss from litterbags, labelled material in the field, or CO_2 efflux from laboratory incubations or field soils. In temperate systems, it appears that fresh litter is nearly completely incorporated into the soil (Angers et al., 1997; Cotrufo et al., 2015), but the rate of decomposition varies with temperature, moisture, or substrate quality (Cleveland et al., 2014; Conant et al., 2011; Devêvre and Horwáth, 2000; Reichstein et al., 2005; Schmitz et al., 2013; Sierra et al., 2015). Varying the rate of litter decomposition may alter SOM accumulation via changes in microbial composition, microbial efficiency, or adsorption/desorption rates (Conant et al., 2011; Dijkstra et al., 2011).

Persistence of SOM increases with physical protection in aggregates or mineral association (Kögel-Knabner et al., 2008; Lehmann and Kleber, 2015; Marin-Spiotta et al., 2009; Six et al., 2000a). Microbially-derived compounds are the majority of mineral-associated SOM and have been shown to be critical for soil aggregation (Chaney and Swift, 1986; Cotrufo et al., 2013; Grandy and Neff, 2008; Kallenbach et al., 2015; Plaza et al., 2013; Soong et al., 2015; Tisdall and Oades, 1982). Therefore, microbial carbon use efficiency (CUE, the proportion of C substrate assimilated rather than respired by microbes), as well as total microbial biomass C (MBC) during decomposition, may determine the degree of physical protection of SOM and subsequent persistence. However, high MBC was associated with losses of mineral-associated C unless litter C:N ratio was low (Finn et al., 2016), and shoot C may be more efficiently mineral-associated than root C (Lavallee et al., 2018), highlighting need the for investigations into interactions between microbial activity and mineral association under varying conditions.

Despite the importance of microbial activity for aggregation, little is known about how temperature and moisture regimes affect soil aggregate stability (Hamdi et al., 2013; Prescott, 2010; Zhou et al., 2014). Stable aggregates declined along a warming gradient in a subarctic grassland (Poeplau et al., 2017), but temperature had no effect on C in any soil fraction in a field warming experiment on an arable soil (Grunwald et al., 2017). For moisture, the amplitude and history of dry-wet cycles may be more

important than mean soil water content. Rapid re-wetting, or slaking can be extremely destructive even to stable aggregates as air trapped inside aggregates bursts out once aggregates are submerged in water (Le Bissonnais, 1996; Wu et al., 2017). Antecedent moisture conditions can determine short-term C mineralization (Smith et al., 2017). Dry-wet cycles can reduce aggregate stability due to slaking, probably due to decomposition of labile organic matter, but this effect is not consistent in all soils (Cosentino et al., 2006; Degens and Sparling, 1995). While greater aggregate stability offers longer physical protection for soil C, faster aggregate turnover may allow greater incorporation of litter C into aggregates (Denef et al., 2001; Tisdall and Oades, 1982). This conflicting evidence points to a need to connect microbial processes more directly with soil aggregate formation and stability. Because temperature and moisture vary seasonally, spatially, and with human management, properly constraining their effects on physical C protection is critical to assessing our ability to increase global C stocks (Minasny et al., 2017).

To illuminate fundamental controls on soil C persistence and predict how management and climate change may affect soil C stocks, we subjected the same soil and substrate to varying environmental conditions in a 6-month incubation. Focusing our investigation on providing preliminary evidence for the relationships between microbial activity and physical protection of soil C, our objectives were to evaluate 1) how temperature and moisture manipulations affected microbial biomass, activity, and composition, and 2) how soil aggregate distribution, incorporation of substrate C, and aggregate C chemistry was affected by temperature and moisture or resultant microbial activity. We hypothesized that MBC and activity would decrease with warmer temperatures, leading to less aggregation and incorporation of substrate C. However, we expected that complex C would be more easily broken down under warm conditions, leading to proportionally more simple C.

4.3 Materials and methods

4.3.1 Experimental design

Plano silt loam was collected from the Wisconsin Integrated Cropping Systems Trial's (WICST) 25-year-old continuous corn plots in June 2015 (Posner et al., 1995). Soil was air-dried, sieved to 2 mm, and visible roots and detritus were removed. The $\delta^{13}C$ of this soil was -17.15 ‰. Biomass (*Mimulus ringens* L. and *Verbena hasta* L.) was grown under elevated CO_2 in the University of Wisconsin-Madison Biotron in 2006 (Kao-Kniffin and Zhu, 2013), which led to a depleted $\delta^{13}C$ signature of -42.32‰. Soil was packed in 100-mL plastic specimen cups to a bulk density of 1.20 g cm^{-3}, with 2% biomass by mass incorporated, except in benchmark samples, which received no biomass additions. This amounted to an addition of 1.02 g C, or 8.54 mg C g^{-1} soil. Initial soil + biomass mixtures had 38.8 (±0.06) g C and 2.77 (±0.004) g N kg^{-1} soil. Benchmark jars (no plant biomass added, n=4 per treatment) had 29.3 (±0.06) g C and 2.52 (±0.01) g N kg^{-1} soil. Samples were incubated in a 2 x 2 factorial design with temperature (22 or 30 °C) and moisture (45 and 65% water-filled pore space, WFPS) treatments, referred to hereafter as warm dry, warm moist, cool dry, and cool moist. Incubations were kept loosely covered with lids with two 8-mm holes, which minimizes water loss while preventing headspace buildup of CO_2 to a level which would inhibit microbial respiration (Sanford and Kucharik, 2013). Rate of CO_2 efflux from incubations was measured by sealing jars and circulating gas through a LI-COR infrared gas analyzer (xxMODELxx, LI-COR Biosciences, Lincoln, NE) over 2 to 10 min, with readings every second. A linear and a quadratic model of CO_2 concentration over time was fit for each jar and measurement. The quadratic model fit the data 68% of the time, with an average pseudo-R^2 of 0.97. Linear fluxes were used when the CO_2 efflux did not fit a quadratic model, with an average R^2 of 0.87. Efflux was measured at least 3 times per week for the first 30 days, and twice per week thereafter. Samples were kept within 5% of target WFPS by watering after CO_2 efflux measurements as needed.

After converting flux rates to mass loss of C over time using the Ideal Gas Law (Geisseler et al., 2011; Zibilske, 1994), we calculated cumulative loss of C from incubations by linear interpolation between CO_2 efflux measurements. We estimated plant biomass lost to respiration for each treatment jar separately:

Eqn 1: $C_i - C_{benchmark} = C_{est\ plant\ biomass\ (i)}$

Where C_i is the cumulative C loss for a given jar *i*, $C_{benchmark}$ is the mean cumulative C loss for all benchmark jars of the same treatment as jar *i.* This assumes that there was negligible changes in C loss rates for the soil C upon biomass addition: based on strong correlation between this $C_{est\ plant\ biomass}$ and subsequent analysis of C lost according to $\delta^{13}C$ in sampled soils, we are confident that this assumption is valid and the approach was robust (R^2=0.90, *P*<0.0001, Figure 1). Destructive sampling of 4 samples per treatment (one from each quartile of total C respired) took place at ~30%, 50%, and 60% of plant biomass respired (T_{30}, T_{50} and T_{60}, Table 1). At T_{30} and T_{50}, two jars were used for aggregate fractionation, and two were split for analysis of microbial biomass C (MBC), extracellular enzyme activity (EEA), and 16S RNA gene sequencing. Samples for aggregate fractionation, MBC, and EEA were refrigerated until analysis; samples for sequencing were stored at -80 °C. At T_{60}, all four jars were subjected to all analyses. Subsamples from all jars were analyzed for total C and N on a Flash EA 1112 CN Automatic Elemental Analyzer (Thermo Finnigan, Milan, Italy) at incubation initiation and after sampling.

4.3.2 Microbial assays

Microbial biomass C was determined using the chloroform fumigation direct extraction method (Setia et al., 2012; Vance et al., 1987), with K_2SO_4 diluted to 0.05 M in order to reduce damage to the Shimadzu TOC-V instrument used to determine dissolved organic C concentration in the extractant. The $\delta^{13}C$ of dissolved organic C was determined on a TOC analyzer (O.I. Analytical Model 1030 TOC Analyzer Xylem Analytics, College Station, TX) interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK) with a GD-100 Gas Trap Interface (Graden Instruments) at the UC Davis Stable Isotope Facility. Samples were acidified and purged with He to remove inorganic carbonates, which are negligible at this site (Paul et al., 2001). The activity of extracellular enzymes xylosidase, for α-glucosidase, β-glucosidase, N-acetyl-glucosidase, cellobiohydrolase, phosphatase, and leucine-amino-

peptidase (XYL, AG, BG, NAG, CELL, PHOS and LAP) was determined using a fluorescence assay at room temperature (Bell et al., 2013; Steinweg et al., 2012).

We extracted DNA with a Qiagen (Germantown, MD) DNeasy PowerLyzer PowerSoil Kit. Extraction kit instructions were followed for the DNA extraction protocol using 0.25 g of moist soil. As part of the protocol, samples were lysed on a MP Biomedicals FastPrep-24 5G (Santa Ana, CA) for 45 seconds at 6 m s^{-1}. Samples were amplified in triplicate, targeting the 16S rRNA gene v4 region with 515f and 806r primers (Walters et al., 2015), with barcodes and Illumina sequencing adapters added as per Kozich et al. (2013) (Supplementary Table X). PCR was performed with 12.5 μL Q5 Hot Start High-Fidelity 2X Master mix (New England BioLabs INC., Ipswich, MA), 1.25 μL 515f forward primer (10 μM), 1.25 μL 806r reverse primer (10 μM), 1 μL DNA extract, and 7.75 μL PCR-grade water. The reactions took place on an Eppendorf Mastercycler nexus gradient (Hamburg, Germany) thermal cycler as follows: 98 °C for 2 minutes + (98 °C for 30 seconds + 58 °C for 15 seconds + 72 °C for 10 seconds) x 30 + 72 °C for 2 minutes and 4 °C hold. The PCR amplicon triplicates were pooled, purified and normalized using a SequalPrep Normalization Plate (96) Kit (ThermoFisher Scientific, Waltham, MA). Samples were combined and library cleanup was performed using a Wizard SV Gel and PCR Clean-Up System A9282 (Promega, Madison, WI) according to manufacturer's instructions except for the following two deviations (1) the SV Minicolumn incubation and centrifugation (steps 5.A.2-5.A.3) steps were repeated twice for each sample, and (2) nuclease-free water application was divided into 30 μL and 20 μL increments with the incubation step and centrifuge step after each addition (step 5.A.6). The pooled library was submitted to the UW Madison Biotechnology Center (UW-Madison, WI) for 2x250 paired end Illumina MiSeq sequencing.

4.3.3 Aggregate fractionation

Aggregates were separated by size using a wet-sieving procedure as outlined by Elliott (1986), followed by macroaggregate dispersal on a shaker as described by Six et al. (1999). We isolated six soil fractions: macroaggregates (>250 μm), microaggregates (53 to 250 μm), silt & clay (<53 μm), and from within macroaggregates, coarse particulate organic matter (cPOM, >250 μm), occluded microaggregates (53 to 250 μm), and occluded silt & clay (<53 μm). All fractions were analyzed for total C and N on an elemental analyzer (Flash EA1112, Thermo Electron Corp., Milan, Italy) as well as $\delta^{13}C$. Isotopic composition of soil C was determined at the UC-Davis Stable Isotope Facility using a Thermo GC-C-IRMS composed of a Trace GC Ultra gas chromatograph (Thermo Electron Corp, Milan, Italy) coupled to a Delta V Advantage isotope ratio mass spectrometer through a GC/C-III interface (Thermo Electron Corp, Breman, Germany).

4.3.4 Organic C chemistry

The molecular composition of C in aggregate fractions from each sample and the initial soil+biomass mixture, were characterized by electrospray ionization (ESI) coupled with Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR). Sequential extractions with water, methanol and chloroform were performed according to Tfaily et al. (2015). Water and methanol extractions were directly injected into a 21 Tesla Bruker SolariX FT-ICR spectrometer. Chemical formulas were assigned using in-house software, with a signal to noise (S/N) > XX, and error XX, taking into consideration the presence of C, H, O, N, S, and P. Spectra were classified into eight FTI-ICR compound classes, based on O/C and H/C counts: lipids ($0 < O/C \leq 0.3$, $1.5 \leq H/C \leq 2.5$), unsaturated hydrocarbons ($0 < O/C \leq 0.125$, $0.8 \leq H/C < 1.5$), condensed hydrocarbons ($0 < O/C \leq 0.95$, $0.2 \leq H/C < 0.8$), proteins ($0.3 < O/C \leq 0.55$, $1.5 \leq H/C \leq 2.3$), amino sugars ($.55 < O/C \leq 0.7$, $1.5 \leq H/C \leq 2.2$), carbohydrates ($0.7 < O/C \leq 1.5$, $1.5 \leq H/C \leq 2.5$), lignins ($0.125 < O/C \leq 0.65$, $0.8 \leq H/C < 1.5$), tannins ($0.65 < O/C \leq 1.1$, $0.8 \leq H/C < 1.5$) (see van Krevlan diagram, Supplementary Figure 1). These metrics for classification neglect molecular

structure but do represent broadly different compound classes. Processing code can be found at: https://github.com/ktoddbrown/ FTICR_Processing.

4.3.5 Statistical Analysis

The effects of temperature, moisture, sample date and their interactions on microbial biomass C, EEA, total C and N, proportion of plant C, bacterial phyla, FT-ICR compound classes, aggregate mass proportion and aggregate C and N were evaluated using the *aov* function in R (R version 3.3, R Core Team 2016). Control data were excluded from this analysis, but a second model added the effects of biomass addition for response variables at T_{60}. This allowed us to evaluate whether trends related to temperature and moisture were robust regardless of biomass addition. Enzyme activities were log-transformed to meet assumptions of the normal distribution. Where a significant interaction with date occurred, the effects of temperature and moisture within a given date are presented separately. To interpret patterns in relative intensities of individual peaks measured by FT-ICR and relative abundances of bacterial OTUs, ordinations were created with *metaMDS* in the vegan package using Bray-Curtis distance matrices. PERMANOVA (*adonis*, vegan) was used to determine significant differences by temperature, moisture sample date, and biomass addition within the distance matrices. The *envfit* function from the vegan package (R Core Team, 2016) was used to evaluate the relationship between response variables and bacterial or molecular composition.

4.4 Results

4.4.1 Temperature and moisture effects on C mineralization, microbial biomass, activity and composition

Overall, the incubation showed a stronger impact of temperature than moisture on microbial biomass, activity, and composition. Warm temperatures increased mineralization rates at T_{30} and T_{50} in both biomass and control jars (Table 1), and warm moist jars had a greater C mineralization rate than warm dry jars at only in T_{30} biomass-added jars. At T_{60}, temperature did not affect mineralization rate, but the

cumulative C respired was greater in warm biomass-added jars than cool dry biomass-added jars. In control jars at T_{60}, cumulative C was greater in warm than cool jars. The minimal effects of moisture suggest moisture was sufficient for microbial C mineralization irrespective of temperature treatment.

Microbial biomass responded to both temperature and moisture (Figure 3). At T_{30} and T_{50}, MBC was greater in cool dry jars than all other treatments, but at T_{60}, MBC was greater in all cool jars. The effect of temperature was consistent irrespective of biomass addition, but control jars had lower overall MBC than biomass-added jars.

Microbial activity as measured by EEA responded differently to temperature and moisture. The enzymes BG, NAG, PHOS, and XYL, used to depolymerize C compounds, were elevated at warm temperatures at T_{30}, whereas the enzyme LAP, which releases N from the protein leucine, was elevated in cool jars at T_{50} and T_{60} (Figure 2, Table 2). Moisture had a mixed effect on EEA depending on enzyme and sample date. When biomass was added, moist jars had higher BG, CELL, PHOS at T_{50}, but PHOS and NAG were elevated in dry jars at T_{30}. In control jars, only PHOS activity was elevated in warm treatments (Figure 2, Supplemental Table 2). However, NAG and LAP activity at T_{60} were greater in control than biomass added jars (Figure 2). Since no nutrients were added to control jars, production of NAG and LAP may have been required for microbes to acquire N.

Bacterial communities clustered according to sample date, temperature treatment, and biomass addition, all of which were significant according to PERMANOVA ($P<0.01$, Figure 4, Table 3).

4.4.2 Soil aggregation and C chemistry

Moisture had a strong effect on soil aggregate distribution, with drier conditions increasing aggregation and associated C and N. Dry jars showed significantly larger proportions of soil in macroaggregates, occluded microaggregates and occluded silt and clay, with proportionally less soil in silt and clay (Table 4, Figure 5). The proportion of soil in coarse POM, however, was significantly increased in cool jars at T_{30}

and warm jars at T_{50}. Biomass addition increased the proportion of soil in aggregates, while control jars had elevated silt and clay (Supplemental Table 2).

Aggregate C and N content was increased while fine fraction C and N decreased in dry jars (Figure 5, Table 4, Supplemental Table 2). Across samples and biomass addition, dry jars contained greater occluded microaggregate and less silt and clay C. Occluded microaggregate and macroaggregate N were also greater under dry conditions, with correspondingly less silt and clay N. In control jars, occluded silt and clay C and N were also greater under dry conditions. Coarse POM C and N, macroaggregate N, occluded microaggregate N, and total C decreased during the incubation, but silt and clay N increased. Biomass addition decreased the C and N content of silt and clay and had no effect on microaggregate C or N, but increased C and N in all other fractions.

Across fractions, lipids (64%) were the most abundant, followed by proteins (11%), lignin (10%) and unknown compounds (8%). Amino sugars, carbohydrates, condensed hydropcarbons, and tannins were found consistently across fractions in low abundances (<2% each) (Figure 6). Both silt and clay fractions were depleted in carbohydrates and depleted in proteins relative to other fractions. Free silt and clay spiked in condensed hydrocarbons, tannins, and unknown compounds at T_{60} accompanied by a drop in relative abundance of lipids and proteins. Across sample dates and treatments, microaggregates were consistently enriched in amino sugars.

The chemical composition of all soil fractions changed over time while the effect of temperature and moisture varied among aggregate fractions (Table 3, Figure 7). In particular, temperature affected chemical composition of macroaggregates, microaggregates, occluded microaggregates, and silt and clay. Moisture affected the chemical composition of only macroaggregates, microagregates, and occluded microaggregates. The chemical composition of coarse POM varied only with sample date.

The overall number of peaks, an indicator of OM richness, declined in macroaggregates and silt and clay after T_{50}, but remained fairly stable in other fractions (Table 5, Figure 8). Treatment also affected OM richness in some aggregate fractions: more peaks found in moist samples in macroaggregates, while more peaks were found in cool samples in silt and clay, and more peaks were found in warm treatments in occluded microaggregates. Treatments did not affect OM richness in occluded silt and clay, microaggregates, or coarse POM.

Proteins and lipids, two of the simple compound classes, were affected by temperature especially in the fine fractions: cool jars had elevated proteins in occluded silt and clay and free silt and clay, and elevated lipids in free silt and clay at the third sample date (Figure 8, Table 5). Lipids were elevated under warm temperatures in occluded silt and clay, however, and amino sugars also increased with warm temperatures in macroaggregates, silt and clay, occluded microaggregates, and coarse POM. Proteins did not vary in macroaggregates or occluded microaggregates. Lipids were elevated in macroaggregates under cool dry conditions and elevated in occluded microaggregates under cool conditions. In microaggregates the effects of temperature and moisture on protein and lipid abundance were highly variable, but cool dry conditions generally elevated proteins and lipids.

Carbohydrates, another simple compound, varied only in larger aggregate fractions, with no significant effect of temperature and moisture in silt and clay or occluded silt and clay (Table 5, Figure 8). Temperature elevated carbohydrates in macroaggregates, occluded microaggregates, and coarse POM. Microaggregates were again more variable, with elevated carbohydrates under wet conditions at T_{30}, dry conditions at T_{50}, and warm conditions at T_{60}.

Complex C compound classes were also more affected by treatments in coarser fractions (Table 5, Figure 8). Tannins and condensed hydrocarbons were elevated under moist conditions, especially early in

decomposition. Lignin and unsaturated hydrocarbons, in contrast, were increased under warm conditions in macroagregates, microaggregates and occluded microaggregates.

4.4.3 Plant C incorporation into soil C pools

Treatments had a much stronger effect on the proportion plant C in MBC than soil C fractions, with a total of up to 60% of plant recovered. Plant C made up 30 to 50% of MBC, varying significantly by temperature, moisture, and date (Figure 9). Incorporation of plant C into MBC was greater in cool, dry treatments and dropped after T_{30} for all treatments except warm moist jars, which maintained the lowest but most stable proportion of plant C in microbial biomass.

Dry conditions increased the proportion of C from plant litter additions in silt and clay (P<0.001, Supplemental Table 2, Supplemental Figure 3). However, silt and clay C stock was smaller under dry conditions (Figure 5), so total plant C stabilized in silt and clay was not different among treatments (data not shown). Treatment did not affect any other aggregate fraction in proportion of C from plant biomass (Supplemental Table 3) or total plant biomass C (data not shown). However, conversion of plant C to macroaggregate, microaggregate C and occluded microaggregate C was more efficient under cool conditions at T_{60} (P<0.05, Figure 10). In other words, after 6 months of incubation, less plant C was respired per unit plant C incorporated into aggregates under cool conditions than warm conditions.

The proportion of plant biomass C in all aggregate fractions decreased over time (Supplemental Figure 3, Table 4). Although the proportion of plant biomass in all fractions declined between T_{30} and T_{50}, only occluded microaggregates significantly declined after T_{50}, indicating lower rate of plant biomass C respiration and overall greater stability in C pools after ~50% of plant C was respired.

4.5 Discussion

This indicates that the microbial contribution to soil aggregate stability will be mediated by environmental conditions.

Greater efficiency of plant C stabilization under cool dry conditions aligns with previous evidence of increasing CUE with decreased temperature (Devêvre and Horwáth, 2000; Dijkstra et al., 2011; Frey et al., 2013; Steinweg et al., 2008). Increasing microbial biomass per unit C respired represents a pathway for conversion of high-quality (low C:N) plant litter into stable SOC (Creamer et al., 2016; Kallenbach et al., 2015). In addition, microbially-derived C is likely to associate with minerals, as shown by elevated in lipids and proteins in the fine fractions under cool conditions. Other studies have also found that mineral-associated organic matter is primarily labile compounds (Haddix et al., 2016), presumed to be of microbial origin (Grandy and Neff, 2008).

High EEA in our incubations coincided with early high C mineralization rates in warm jars and overall higher C mineralization in biomass-added jars. Although BG has been shown to be sensitive to moisture at 10 to 40% volumetric soil moisture (Steinweg et al., 2012), and hot dry field conditions increased CELL activity (Doyle et al., 2006), under our laboratory conditions substrate diffusion was apparently not limited by moisture and temperature was the primary driver of C cycling enzymes like BG, CELL, and PHOS. Moisture affected XYL, which has been shown to be more sensitive to disturbance than other hydrolytic enzymes, perhaps because it occurs at lower concentrations (Blankinship et al., 2014). As shown in other circumstances (Hargreaves and Hofmockel, 2014), high microbial biomass C did not lead to high EEA, because enzyme production is likely stimulated by substrate shortage while microbial biomass growth or high CUE increase with low C:N substrate availability (Moorhead et al., 2012).

Aggregate C and silt and clay C were both sensitive to temperature and moisture in this experiment, although they represent different modes of physical C protection. The silt and clay C generally consists of microbially-derived compounds tightly bound through electrostatic interactions between minerals

and charged organic matter (Kleber et al., 2007). Macroaggregates contain networks of micro-pores, which are elevated in more complex C (Bailey et al., 2017), and rely on binding from roots and fungal hyphae (Six et al., 2000b), which were probably minimal in the our incubation. Diffusion of substrates and release of soluble C under moist conditions might be expected to reduce both silt and clay and aggregate C; however, we found that only macroaggregate C was reduced in moist conditions. This may be because aggregate C is more loosely bound than silt and clay C and therefor more sensitive to disturbance (Panettieri et al., 2015; Trivedi et al., 2015). Decomposition of cPOM has been shown increase with temperature more than mineral-associated C (Benbi et al., 2014), suggesting that cPOM and aggregate C will be rapidly mineralized if changes in abiotic conditions render them accessible to microbes . Nevertheless, increasing aggregate C is critical for increasing total C stocks, particularly where they have been severely depleted by agricultural disturbance (Minasny et al., 2017; Six et al., 2000a). Our evidence suggests that building aggregate C will be more rapid and feasible under drier conditions.

Although soil aggregate and pore size distribution are known drivers of microbial community structure (Bach et al., 2018; Smith et al., 2017), we found that similar microbial communities only induced aggregation under dry conditions. The effect of moisture on microbial success is mediated by a complex interplay between gas and solute diffusion (Manzoni et al., 2012). Diffusion of substrates may impact aggregate binding as simple substrates are more easily accessed by microbes under moist conditions, leaving aggregates relatively enriched in complex C (Smith et al., 2017). The microbially-derived C compounds that enhance aggregation are ubiquitous in soil, so all the microbial communities present here hold the potential to increase soil aggregation (Plaza et al., 2013). However high-resolution imaging reveals a patchy distribution of microbial necromass and organic matter (Kuzyakov and Blagodatskaya, 2015; Miltner et al., 2012), which may be affected by moisture regime. In addition, the binding ability of these microbial exudates may have been enhanced under drier conditions, leading to more stable aggregates throughout the incubation and regardless of biomass addition. This suggests that while

increasing microbial biomass may increase the pool of C available for mineral association (Grandy and Neff, 2008; Miltner et al., 2012), increasing total biomass is not necessary for increasing aggregate-protected C.

A critical question for predicting soil C stabilization over time is whether conversion of plant litter to stable soil C will be affected by climate regime. Although C mineralization proceeded more rapidly under warm conditions, plant litter C in aggregates was equivalent among temperature and moisture treatments. The priming effect of C addition has also been shown to be the same at warm or cool temperatures (Thiessen et al., 2013), so we believe that primed respiration of native soil C due to litter addition did not differ among treatments. However, greater efficiency of plant C incorporation into aggregates under cool conditions suggests that more plant C will be respired per unit plant C physically protected under warmer conditions, decreasing potential for stabilization of newly incorporated C. In addition, the drastic decrease in aggregation with moisture suggests that wetter conditions will decrease the physical protection of both newly added and native C. It is unclear whether this effect will be stable under varying moisture regimes, as frequency and amplitude of dry-wet cycles are known to impact aggregate stability, but cumulative C mineralization is more closely related to long-term moisture regime than wetting pulses (Borken and Matzner, 2009; Cosentino et al., 2006; Joly et al., 2017).

4.6 Conclusions

We found that while temperature drove microbial composition, activity, and C mineralization, aggregation was enhanced by drier conditions. The complex interactions between diffusion of substrates, microbial physiology, and temperature and moisture effects on the binding strength of various C compounds, dictate that the relationship between temperature or moisture and C protection is unlikely to be linear on the microscale, despite evidence that temperature and moisture drive soil C protection on the global scale. We showed that while microbial necromass and exudates may be critical

for aggregate binding, similar microbial communities' ability to increase physically protected C will be limited by moisture regime. Given evidence for shifting precipitation regimes in many regions due to climate change, our predictions of global C stocks should be adjusted to account for these anomalies. More research is needed to determine the effects of precipitation frequency and intensity on aggregate distribution and efficiency of conversion of plant C to stable soil C.

4.7 Acknowledgments

The authors are grateful to Kelsey Johnson and Megan Lipke for laboratory assistance, to Bill Hickey for lab space, and to Rosey Chu, Malak Tfaily, and Nicola Tolic at Environmental Molecular Sciences Laboratory for assistance with FT-ICR data. Funding was provided in part by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DEFC0207ER64494).

4.8 References

Angers, D.., Recous, S., Aita, C., 1997. Fate of carbon and nitrogen in water-stable aggregates during decomposition of 13C15N-labelled wheat straw in situ. European Journal of Soil Science 48, 295–300. doi:10.1111/j.1365-2389.1997.tb00549.x

Bach, E.M., Williams, R.J., Hargreaves, S.K., Yang, F., Hofmockel, K.S., 2018. Greatest soil microbial diversity found in micro-habitats. Soil Biology and Biochemistry 118, 217–226. doi:10.1016/J.SOILBIO.2017.12.018

Bailey, V.L., Smith, A.P., Tfaily, M., Fansler, S.J., Bond-Lamberty, B., 2017. Differences in soluble organic carbon chemistry in pore waters sampled from different pore size domains. Soil Biology and Biochemistry 107, 133–143. doi:10.1016/J.SOILBIO.2016.11.025

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 e50961. doi:10.3791/50961

Benbi, D.K., Boparai, A.K., Brar, K., 2014. Decomposition of particulate organic matter is more sensitive to temperature than the mineral associated organic matter. Soil Biology and Biochemistry 70, 183–192. doi:10.1016/J.SOILBIO.2013.12.032

Blankinship, J.C., Becerra, C.A., Schaeffer, S.M., Schimel, J.P., 2014. Separating cellular metabolism from exoenzyme activity in soil organic matter decomposition. Soil Biology and Biochemistry 71, 68–75. doi:10.1016/j.soilbio.2014.01.010

Borken, W., Matzner, E., 2009. Reappraisal of drying and wetting effects on C and N mineralization and fluxes in soils. Global Change Biology 15, 808–824. doi:10.1111/j.1365-2486.2008.01681.x

Chaney, K., Swift, R.S., 1986. Studies on aggregate stability. I. Re-formation of soil aggregates. Journal of Soil Science 37, 329–335. doi:10.1111/j.1365-2389.1986.tb00035.x

Cleveland, C.C., Reed, S.C., Keller, A.B., Nemergut, D.R., O'Neill, S.P., Ostertag, R., Vitousek, P.M., 2014. Litter quality versus soil microbial community controls over decomposition: a quantitative analysis. Oecologia 174, 283–94. doi:10.1007/s00442-013-2758-9

Conant, R.T., Ryan, M.G., Ågren, G.I., Birge, H.E., Davidson, E.A., Eliasson, P.E., Evans, S.E., Frey, S.D., Giardina, C.P., Hopkins, F.M., Hyvönen, R., Kirschbaum, M.U.F., Lavallee, J.M., Leifeld, J., Parton, W.J., Megan Steinweg, J., Wallenstein, M.D., Martin Wetterstedt, J. å., Bradford, M.A., 2011. Temperature and soil organic matter decomposition rates - synthesis of current knowledge and a way forward. Global Change Biology 17, 3392–3404. doi:10.1111/j.1365-2486.2011.02496.x

Cosentino, D., Chenu, C., Le Bissonnais, Y., 2006. Aggregate stability and microbial community dynamics under drying–wetting cycles in a silt loam soil. Soil Biology and Biochemistry 38, 2053–2062. doi:10.1016/J.SOILBIO.2005.12.022

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

Cotrufo, M.F., Wallenstein, M.D., Boot, C.M., Denef, K., Paul, E., 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? Global Change Biology 19, 988–95. doi:10.1111/gcb.12113

Creamer, C.A., Jones, D.L., Baldock, J.A., Rui, Y., Murphy, D. V., Hoyle, F.C., Farrell, M., 2016. Is the fate of glucose-derived carbon more strongly driven by nutrient availability, soil texture, or microbial biomass size? Soil Biology and Biochemistry 103, 201–212. doi:10.1016/j.soilbio.2016.08.025

Degens, B.P., Sparling, G.P., 1995. Repeated wet-dry cycles do not accelerate the mineralization of organic C involved in the macro-aggregation of a sandy loam soil. Plant and Soil 175, 197–203. doi:10.1007/BF00011355

Denef, K., Six, J., Bossuyt, H., Frey, S.D., Elliott, E.T., Merckx, R., Paustian, K., 2001. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biology and Biochemistry 33, 1599–1611.

Devêvre, O.C., Horwáth, W.R., 2000. Decomposition of rice straw and microbial carbon use efficiency under different soil temperatures and moistures. Soil Biology and Biochemistry 32, 1773–1785. doi:10.1016/S0038-0717(00)00096-1

Dijkstra, P., Thomas, S.C., Heinrich, P.L., Koch, G.W., Schwartz, E., Hungate, B.A., 2011. Effect of temperature on metabolic activity of intact microbial communities: Evidence for altered metabolic pathway activity but not for increased maintenance respiration and reduced carbon use efficiency.

Soil Biology and Biochemistry 43, 2023–2031. doi:10.1016/j.soilbio.2011.05.018

Doyle, J., Pavel, R., Barness, G., Steinberger, Y., 2006. Cellulase dynamics in a desert soil. Soil Biology and Biochemistry 38, 371–376. doi:10.1016/J.SOILBIO.2005.05.017

Elliott, E.T., 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Science Society of America Journal 50, 627–633.

Finn, D., Page, K., Catton, K., Kienzle, M., Robertson, F., Armstrong, R., Dalal, R., 2016. Ecological stoichiometry controls the transformation and retention of plant-derived organic matter to humus in response to nitrogen fertilisation. Soil Biology and Biochemistry 99, 117–127. doi:10.1016/j.soilbio.2016.05.006

Frey, S.D., Lee, J., Melillo, J.M., Six, J., 2013. The temperature response of soil microbial efficiency and its feedback to climate. Nature Climate Change 3, 395–398. doi:10.1038/nclimate1796

Geisseler, D., Horwath, W.R., Scow, K.M., 2011. Soil moisture and plant residue addition interact in their effect on extracellular enzyme activity. Pedobiologia 54, 71–78. doi:10.1016/j.pedobi.2010.10.001

Grandy, A.S., Neff, J.C., 2008. Molecular C dynamics downstream: the biochemical decomposition sequence and its impact on soil organic matter structure and function. The Science of the Total Environment 404, 297–307. doi:10.1016/j.scitotenv.2007.11.013

Grunwald, D., Kaiser, M., Junker, S., Marhan, S., Piepho, H.-P., Poll, C., Bamminger, C., Ludwig, B., 2017. Influence of elevated soil temperature and biochar application on organic matter associated with aggregate-size and density fractions in an arable soil. Agriculture, Ecosystems & Environment 241, 79–87. doi:10.1016/J.AGEE.2017.02.029

Haddix, M.L., Paul, E.A., Cotrufo, M.F., 2016. Dual, differential isotope labeling shows the preferential movement of labile plant constituents into mineral-bonded soil organic matter. Global Change

Biology 22, 2301–2312. doi:10.1111/gcb.13237

Hamdi, S., Moyano, F., Sall, S., Bernoux, M., Chevallier, T., 2013. Synthesis analysis of the temperature sensitivity of soil respiration from laboratory studies in relation to incubation methods and soil conditions. Soil Biology and Biochemistry 58, 115–126. doi:10.1016/j.soilbio.2012.11.012

Hargreaves, S.K., Hofmockel, K.S., 2014. Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. Biogeochemistry 117, 67–79. doi:10.1007/s10533-013-9893-6

Joly, F.-X., Kurupas, K.L., Throop, H.L., 2017. Pulse frequency and soil-litter mixing alter the control of cumulative precipitation over litter decomposition. Ecology. doi:10.1002/ecy.1931

Kallenbach, C.M., Grandy, A.S., Frey, S.D., Diefendorf, A.F., 2015. Microbial physiology and necromass regulate agricultural soil carbon accumulation. Soil Biology and Biochemistry 91, 279–290. doi:10.1016/j.soilbio.2015.09.005

Kao-Kniffin, J., Zhu, B., 2013. A microbial link between elevated CO2 and methane emissions that is plant species-specific. Microbial Ecology 66, 621–629. doi:10.1007/s00248-013-0254-8

Kleber, M., Sollins, P., Sutton, R., 2007. A conceptual model of organo-mineral interactions in soils: Self-assembly of organic molecular fragments into zonal structures on mineral surfaces. Biogeochemistry 85, 9–24. doi:10.1007/s10533-007-9103-5

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

Kuzyakov, Y., Blagodatskaya, E., 2015. Microbial hotspots and hot moments in soil: Concept &

review. Soil Biology and Biochemistry 83, 184–199. doi:10.1016/J.SOILBIO.2015.01.025

Lavallee, J.M., Conant, R.T., Paul, E.A., Cotrufo, M.F., 2018. Incorporation of shoot versus root-derived 13C and 15N into mineral-associated organic matter fractions: results of a soil slurry incubation with dual-labelled plant material. Biogeochemistry 137, 379–393. doi:10.1007/s10533-018-0428-z

Le Bissonnais, Y., 1996. Aggregate stability and assessment of soil crustability and erodibility: I. Theory and methodology. European Journal of Soil Science 47, 425–437. doi:10.1111/j.1365-2389.1996.tb01843.x

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68. doi:10.1038/nature16069

Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist. doi:10.1111/j.1469-8137.2012.04225.x

Marin-Spiotta, E., Silver, W.L., Swanston, C.W., Ostertag, R., 2009. Soil organic matter dynamics during 80 years of reforestation of tropical pastures. Global Change Biology 15, 1584–1597. doi:10.1111/j.1365-2486.2008.01805.x

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

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

292, 59–86. doi:10.1016/J.GEODERMA.2017.01.002

Moorhead, D.L., Lashermes, G., Sinsabaugh, R.L., 2012. A theoretical model of C- and N-acquiring exoenzyme activities, which balances microbial demands during decomposition. Soil Biology and Biochemistry 53, 133–141. doi:10.1016/j.soilbio.2012.05.011

Panettieri, M., Berns, A.E., Knicker, H., Murillo, J.M., Madejón, E., 2015. Evaluation of seasonal variability of soil biogeochemical properties in aggregate-size fractioned soil under different tillages. Soil and Tillage Research 151, 39–49. doi:10.1016/j.still.2015.02.008

Paul, E.A., Collins, H.P., Leavitt, S.W., 2001. Dynamics of resistant soil carbon of Midwestern agricultural soils measured by naturally occurring 14C abundance. Geoderma 104, 239–256.

Plaza, C., Courtier-Murias, D., Fernández, J.M., Polo, A., Simpson, A.J., 2013. Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: A central role for microbes and microbial by-products in C sequestration. Soil Biology and Biochemistry 57, 124–134. doi:10.1016/j.soilbio.2012.07.026

Poeplau, C., Kätterer, T., Leblans, N.I.W., Sigurdsson, B.D., 2017. Sensitivity of soil carbon fractions and their specific stabilization mechanisms to extreme soil warming in a subarctic grassland. Global Change Biology 23, 1316–1327. doi:10.1111/gcb.13491

Prescott, C.E., 2010. Litter decomposition: what controls it and how can we alter it to sequester more carbon in forest soils? Biogeochemistry 101, 133–149. doi:10.1007/s10533-010-9439-0

R Core Team, 2016. R: a Language and Environment for Statistical Computing.

Reichstein, M., Subke, J.-A., Angeli, A.C., Tenhunen, J.D., 2005. Does the temperature sensitivity of decomposition of soil organic matter depend upon water content, soil horizon, or incubation time? Global Change Biology 11, 1754–1767. doi:10.1111/j.1365-2486.2005.001010.x

Sanford, G.R., Kucharik, C.J., 2013. Effect of methodological consideration on soil carbon parameter estimates obtained via the acid hydrolysis-incubation method. Soil Biology and Biochemistry 67, 295–305. doi:10.1016/j.soilbio.2013.09.002

Schmitz, O.J., Bradford, M.A., Strickland, M.S., Hawlena, D., 2013. Linking predation risk, herbivore physiological stress and microbial decomposition of plant litter. Journal of Visualized Experiments : JoVE e50061. doi:10.3791/50061

Setia, R., Verma, S.L., Marschner, P., 2012. Measuring microbial biomass carbon by direct extraction – Comparison with chloroform fumigation-extraction. European Journal of Soil Biology 53, 103–106. doi:10.1016/j.ejsobi.2012.09.005

Sierra, C.A., Trumbore, S.E., Davidson, E.A., Vicca, S., Janssens, I., 2015. Sensitivity of decomposition rates of soil organic matter with respect to simultaneous changes in temperature and moisture. Journal of Advances in Modeling Earth Systems 7, 335–356. doi:10.1002/2014MS000358

Six, J., Elliott, E.T., Paustian, K., 2000a. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biology & Biochemistry 32, 2099–2103. doi:10.1016/S0038-0717(00)00179-6

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

Six, J., Paustian, K., Elliott, E.T., Combrink, C., 2000b. Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal 64, 681–689. doi:10.2136/sssaj2000.642681x

Smith, A.P., Bond-Lamberty, B., Benscoter, B.W., Tfaily, M.M., Hinkle, C.R., Liu, C., Bailey, V.L., 2017. Shifts in pore connectivity from precipitation versus groundwater rewetting increases soil carbon

loss after drought. Nature Communications 8, 1335. doi:10.1038/s41467-017-01320-x

Soong, J.L., Parton, W.J., Calderon, F., Campbell, E.E., Cotrufo, M.F., 2015. A new conceptual model on the fate and controls of fresh and pyrolized plant litter decomposition. Biogeochemistry. doi:10.1007/s10533-015-0079-2

Steinweg, J.M., Dukes, J.S., Wallenstein, M.D., 2012. Modeling the effects of temperature and moisture on soil enzyme activity: Linking laboratory assays to continuous field data. Soil Biology and Biochemistry 55, 85–92. doi:10.1016/j.soilbio.2012.06.015

Steinweg, J.M., Plante, A.F., Conant, R.T., Paul, E.A., Tanaka, D.L., 2008. Patterns of substrate utilization during long-term incubations at different temperatures. Soil Biology and Biochemistry 40, 2722–2728. doi:10.1016/j.soilbio.2008.07.002

Tfaily, M.M., Chu, R.K., Tolić, N., Roscioli, K.M., Anderton, C.R., Paša-Tolić, L., Robinson, E.W., Hess, N.J., 2015. Advanced Solvent Based Methods for Molecular Characterization of Soil Organic Matter by High-Resolution Mass Spectrometry. Analytical Chemistry 87, 5206–5215. doi:10.1021/acs.analchem.5b00116

Thiessen, S., Gleixner, G., Wutzler, T., Reichstein, M., 2013. Both priming and temperature sensitivity of soil organic matter decomposition depend on microbial biomass – An incubation study. Soil Biology and Biochemistry 57, 739–748. doi:10.1016/j.soilbio.2012.10.029

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. Journal of Soil Science 33, 141–163.

Trivedi, P., Rochester, I.J., Trivedi, C., Van Nostrand, J.D., Zhou, J., Karunaratne, S., Anderson, I.C., Singh, B.K., 2015. Soil aggregate size mediates the impacts of cropping regimes on soil carbon and microbial communities. Soil Biology and Biochemistry 91, 169–181.

doi:10.1016/J.SOILBIO.2015.08.034

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

Wickings, K., Grandy, A.S., Reed, S.C., Cleveland, C.C., 2012. The origin of litter chemical complexity during decomposition. Ecology Letters 15, 1180–1188. doi:10.1111/j.1461-0248.2012.01837.x

Wu, X., Wei, Y., Wang, J., Wang, D., She, L., Wang, J., Cai, C., 2017. Effects of soil physicochemical properties on aggregate stability along a weathering gradient. Catena 156, 205–215. doi:10.1016/j.catena.2017.04.017

Zhou, W., Hui, D., Shen, W., 2014. Effects of soil moisture on the temperature sensitivity of soil heterotrophic respiration: A laboratory incubation study. PLoS ONE 9, e92531. doi:10.1371/journal.pone.0092531

Zibilske, L.M., 1994. Carbon mineralization, in: Weaver, R.W., Angle, S., Bottomley, P.J., Bezdicek, D. (Eds.), Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties SSSA Book Ser. 5. Soil Science Society of America, Madison, WI, pp. 835–864.

4.9 Figures and Tables

Figure 1: Plant C lost at each sampling point (T_{30}, T_{50} and T_{60}, when ~30%, 50% and 60% of added plant biomass had been respired). The x-axis is calculated by linear interpolation between CO_2-C efflux readings (prior to sampling) and the y-axis is calculated from the $\delta^{13}C$ recovered after destructively sampling soil. The solid line is 1:1. Water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").

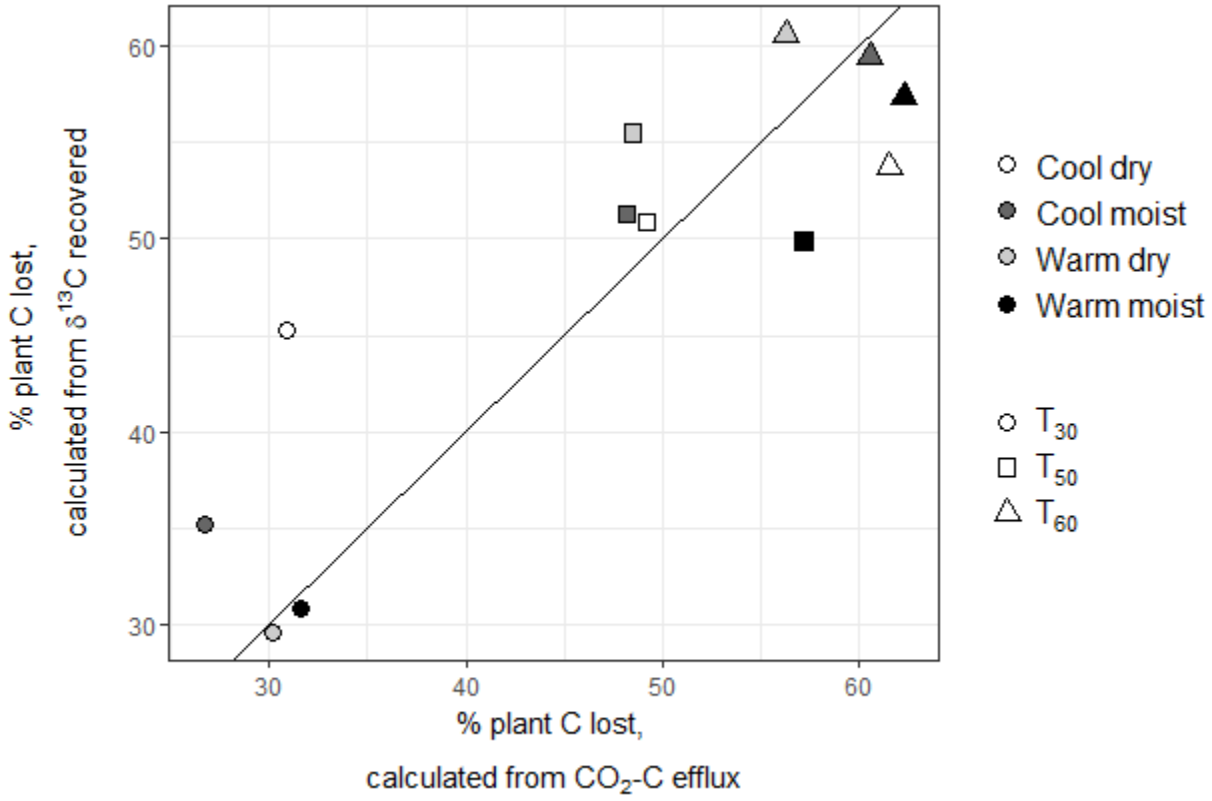


Figure 2: Potential extracellular enzyme activity in a) biomass added and b) control jars for α-glucosidase, β-glucosidase, cellobiosidase, leucine-amino-peptidase, N-acetyl-glucosidase, phosphatase, and xylosidase (AG, BG, CELL, LAP, NAG, PHOS and XYL). Samples 1, 2, and 3 refer to T_{30}, T_{50} and T_{60}, when ~30%, 50% and 60% of added plant biomass had been respired, water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm"). Note that XYL was not measured at T_{50}. For comparisons among treatments, see Table 2.

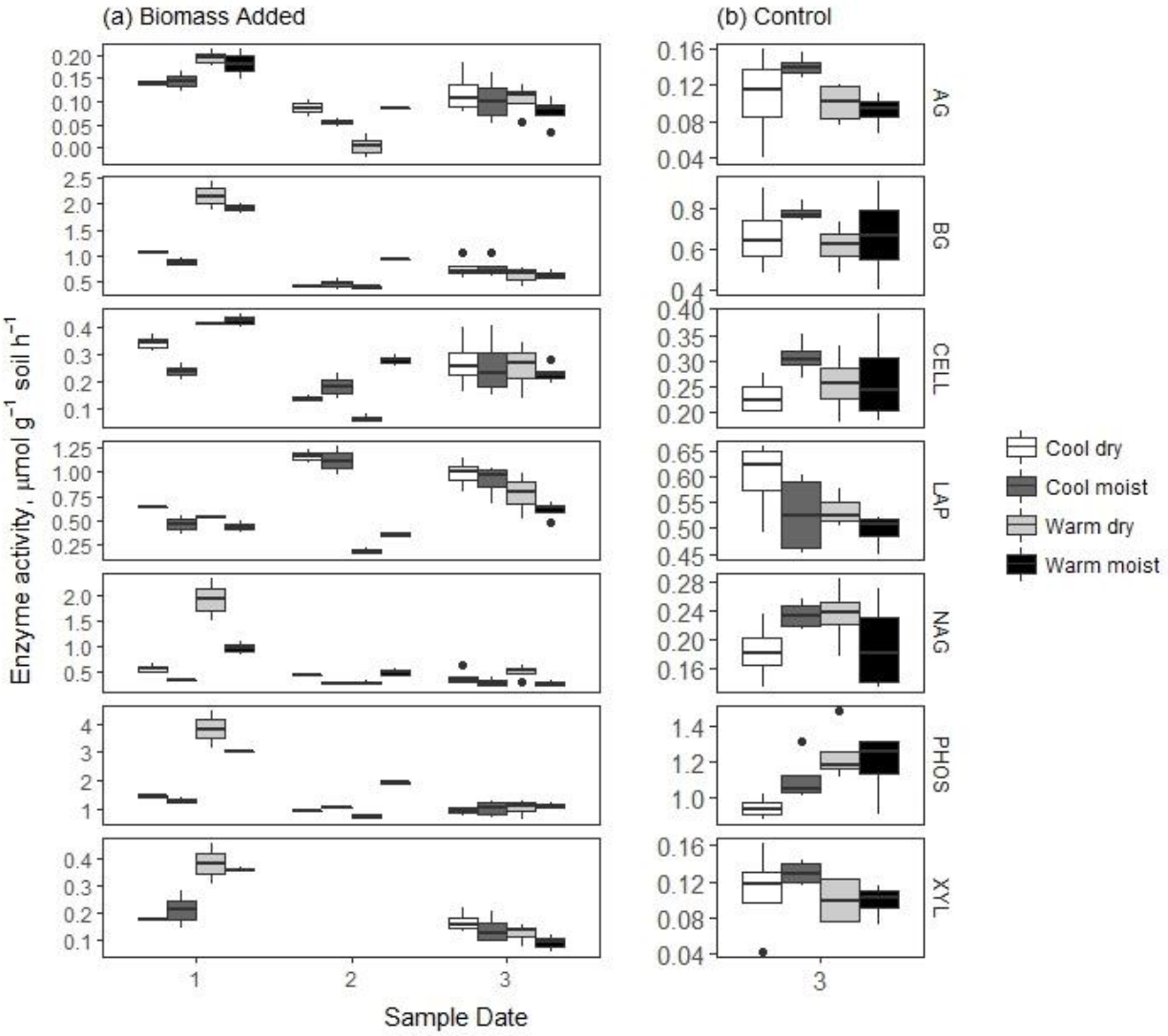


Figure 3. Microbial biomass C in a) biomass added, and b) control jars. Samples 1, 2, and 3 refer to T_{30}, T_{50} and T_{60}, when ~30%, 50% and 60% of added plant biomass had been respired, water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").

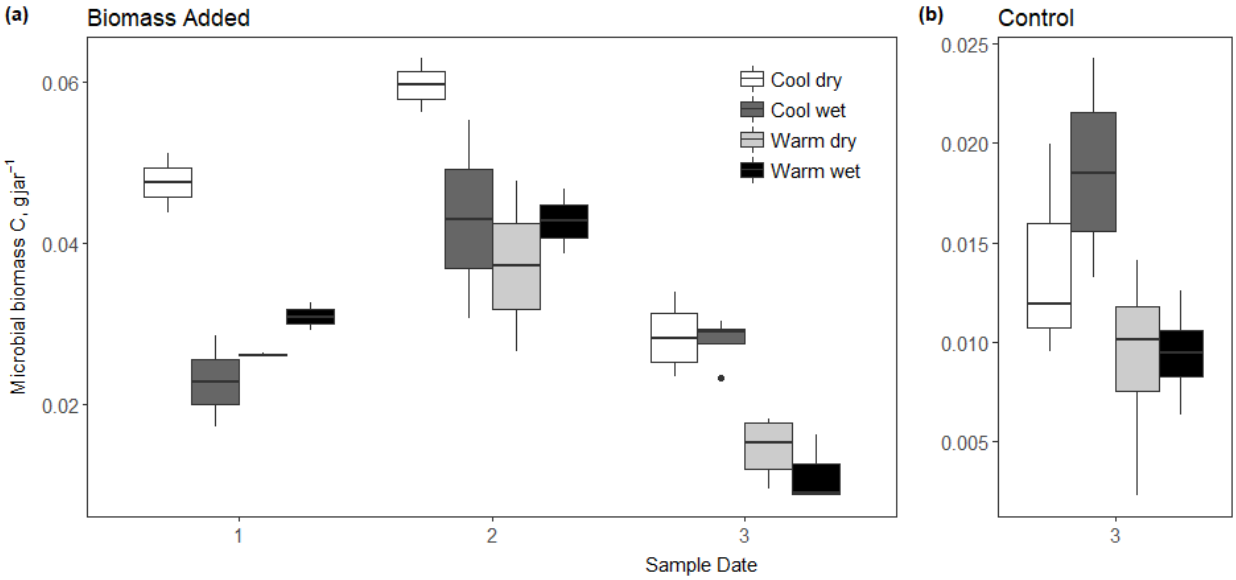


Figure 4: NMDS of Bray-Curtis dissimilarities in 16S microbial community composition. Samples 1, 2, and 3 occurred when 30, 50 and 60% of biomass added had been lost, water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm"). Arrows shown are significant modeled relationships between NMDS axes and potential activity of extracellular enzymes β-glucosidase (BG) and phosphatase (PHOS), moisture and rate of C loss (g C/day) at time of sampling (PERMANOVA $P<0.05$).

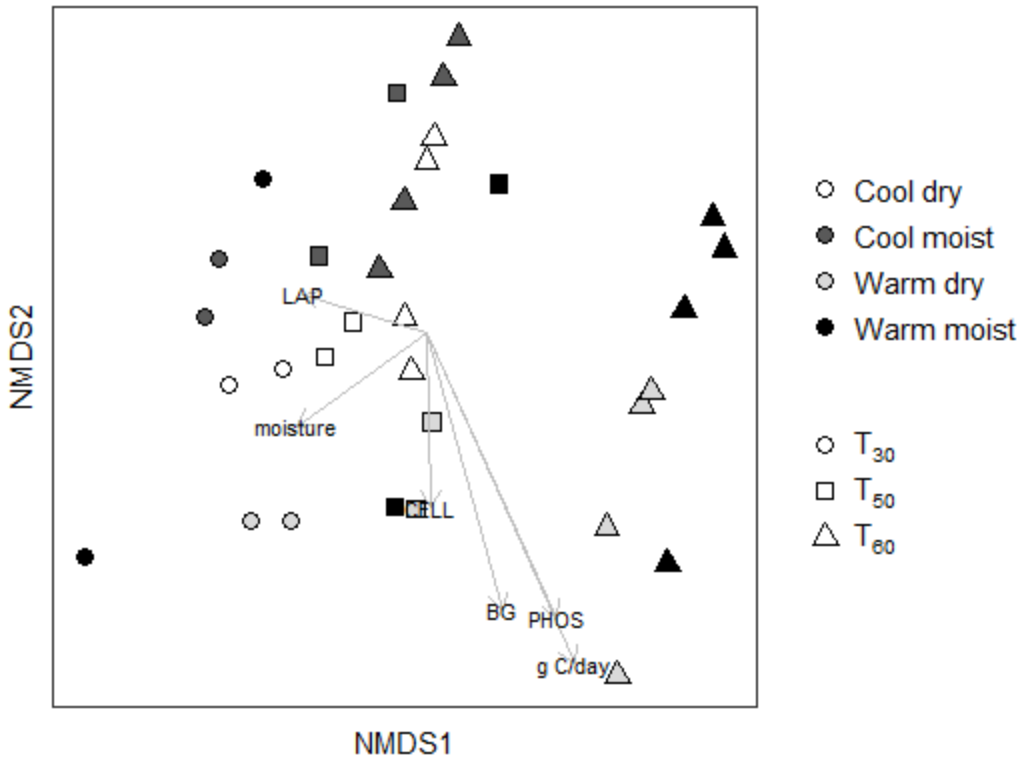


Figure 5. Aggregate (a) distribution, (b) C, and (c) N content at samples T_{30}, T_{50} and T_{60}, when ~30%, 50% and 60% of added plant biomass had been respired. Water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm"). For ANOVA effects of temperature and moisture, see Table 4.

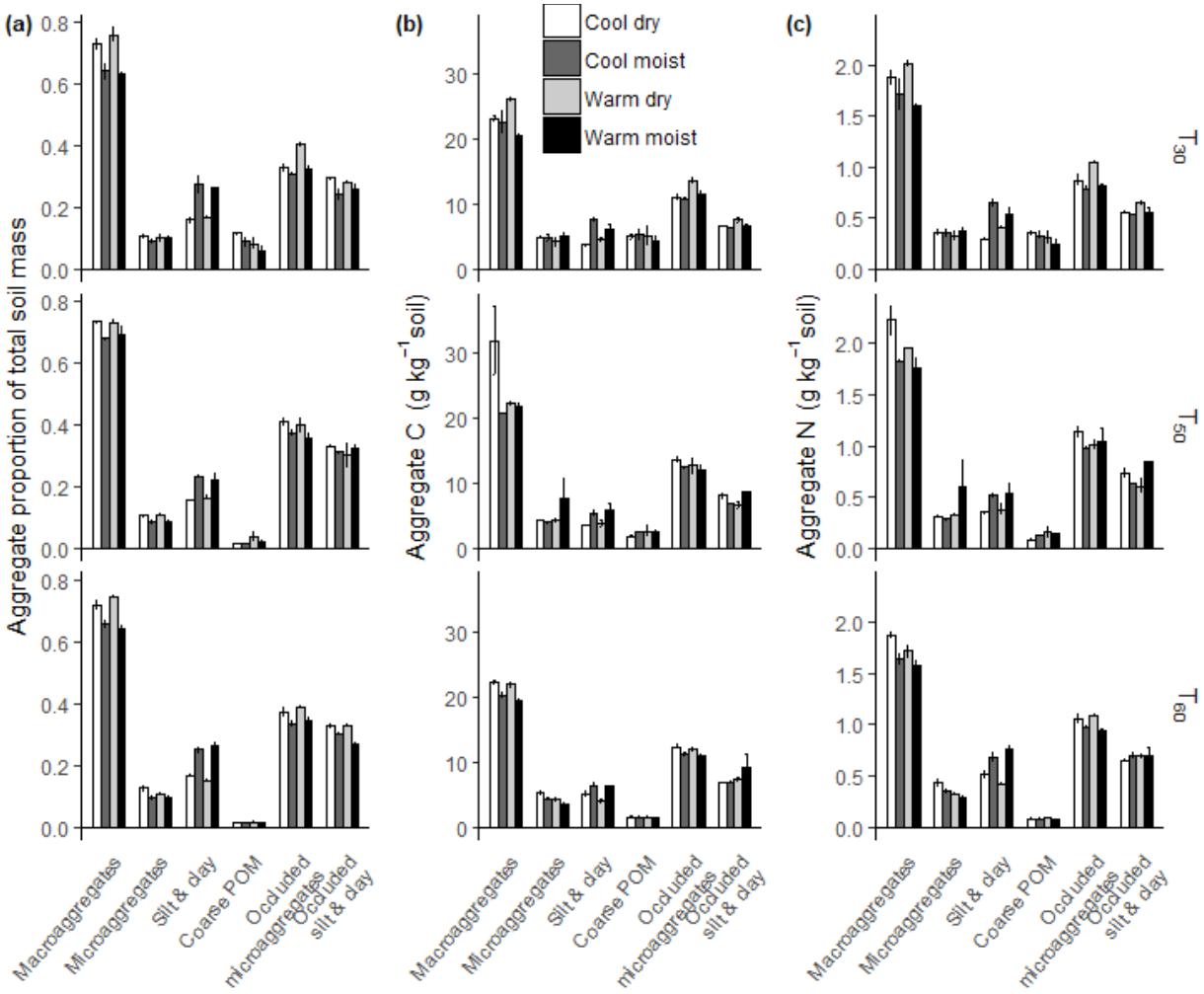


Figure 6. Molecular composition of methanol-extracted carbon in soil aggregate fractions. The relative abundance of Fourier-transform ion cyclotron resonance mass spectrometry defined organic compound classes analyzed in soil aggregates at Samples 1, 2, and 3 refer to T_{30}, T_{50} and T_{60}, when ~30%, 50% and 60% of added plant biomass had been respired.

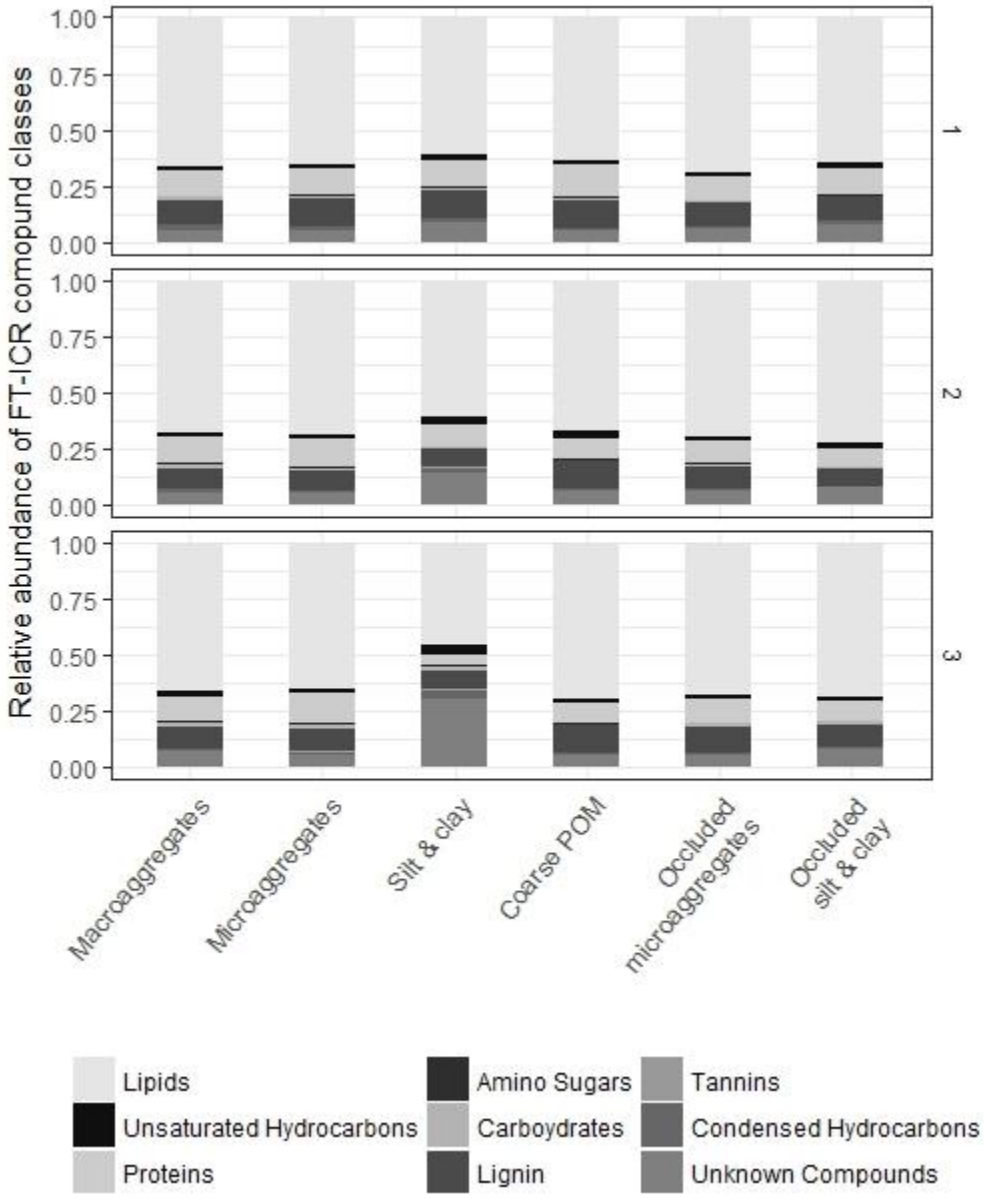


Figure 7. NMDS of FTICR chemistry. Samples 1, 2, and refer to T_{30}, T_{50} and T_{60}, when ~30%, 50% and 60% of added plant biomass had been respired, water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").

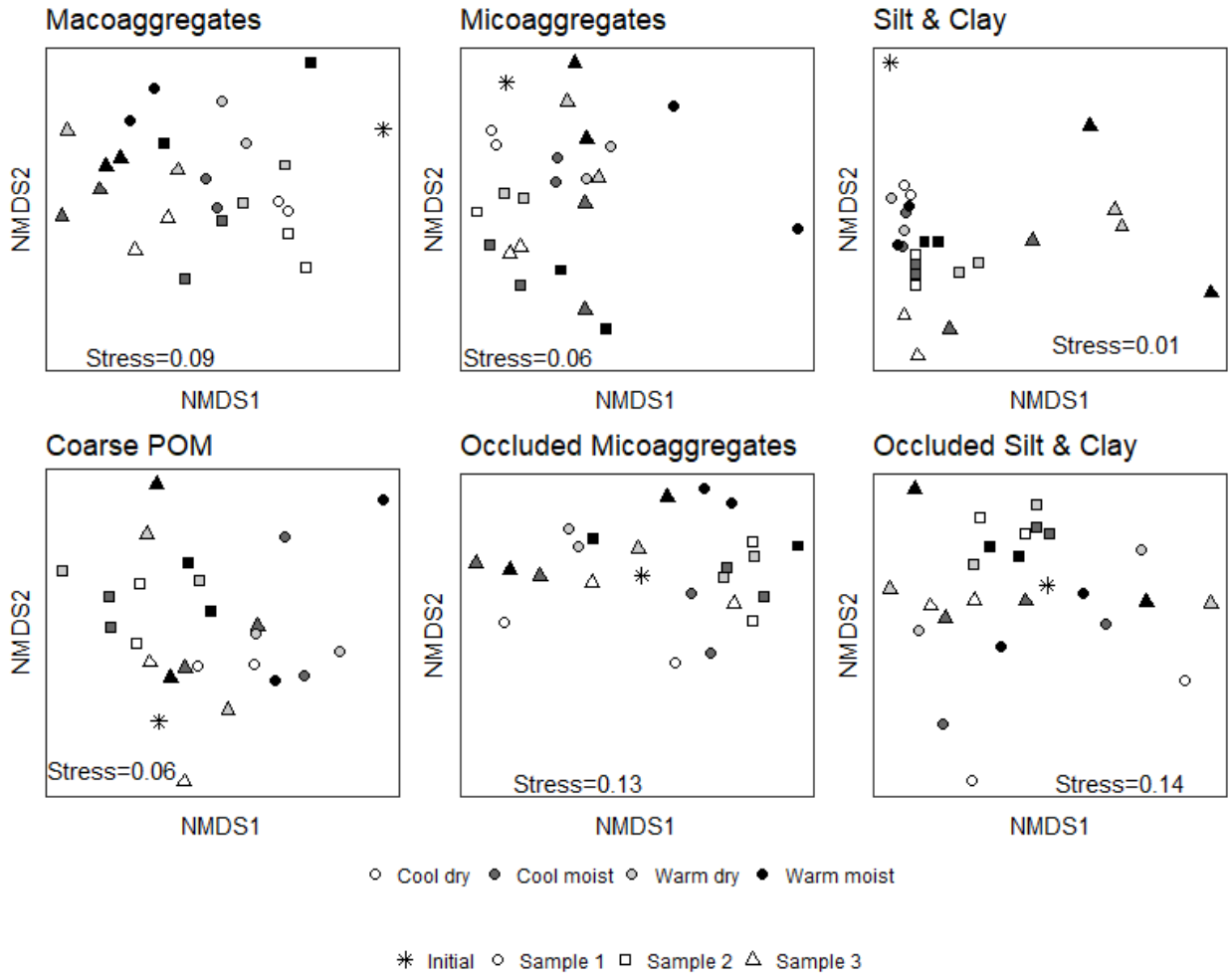


Figure 8: Organic C compound classes in soil aggregates as determined by Fourier-transform ion cyclotron resonance (FT-ICR) mass spectroscopy. Samples 1, 2 and 3 refer to T_{30}, T_{50} and T_{60}, when ~30%, 50% and 60% of added plant biomass had been respired (see Table 1 for details), water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm")..

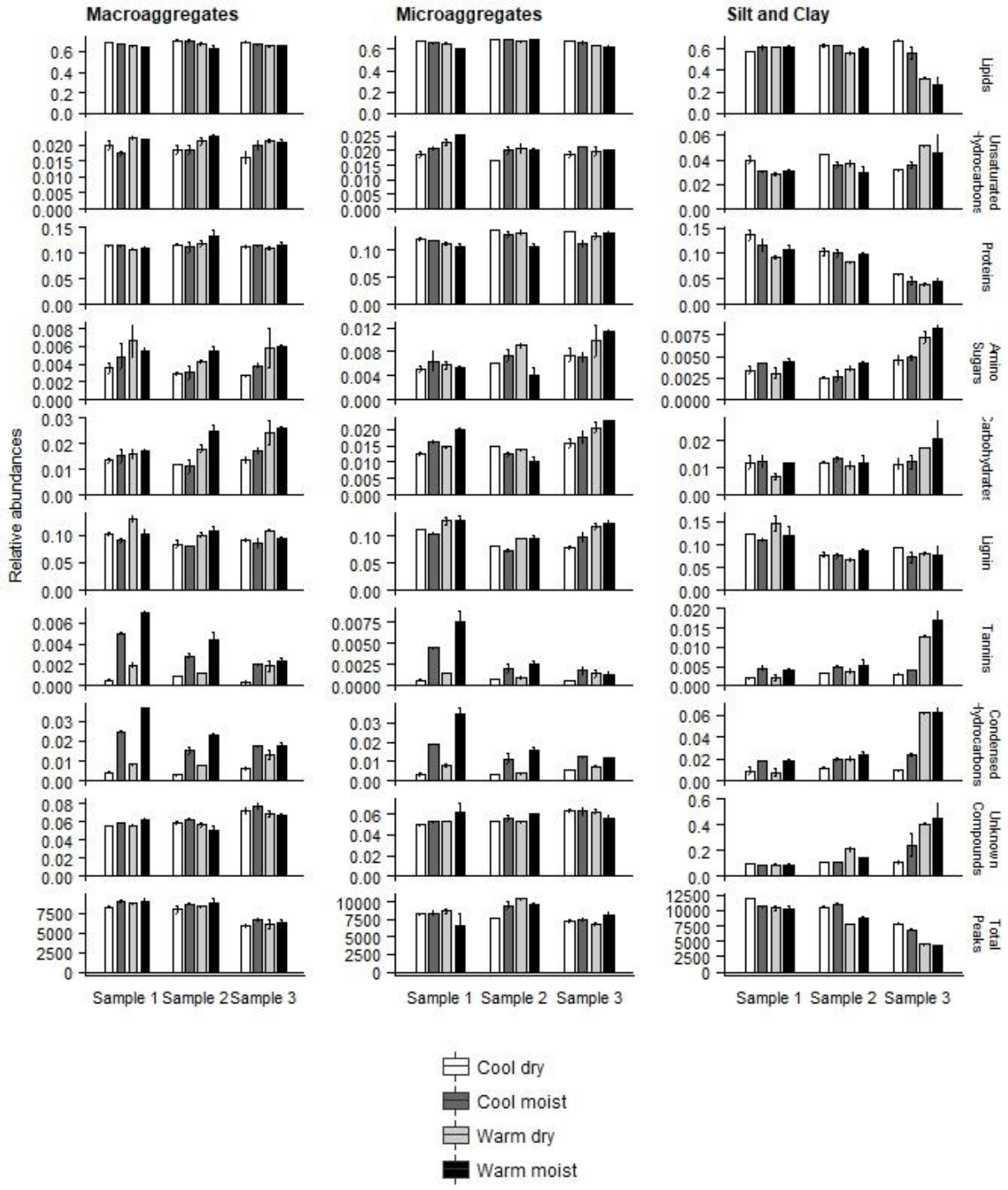


Figure 8 (cont'd): Organic C compound classes in soil aggregates as determined by Fourier-transform ion cyclotron resonance (FT-ICR) mass spectroscopy. Samples 1, 2 and 3 refer to T_{30}, T_{50} and T_{60}, when ~30%, 50% and 60% of added plant biomass had been respired (see Table 1 for details), water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm")..

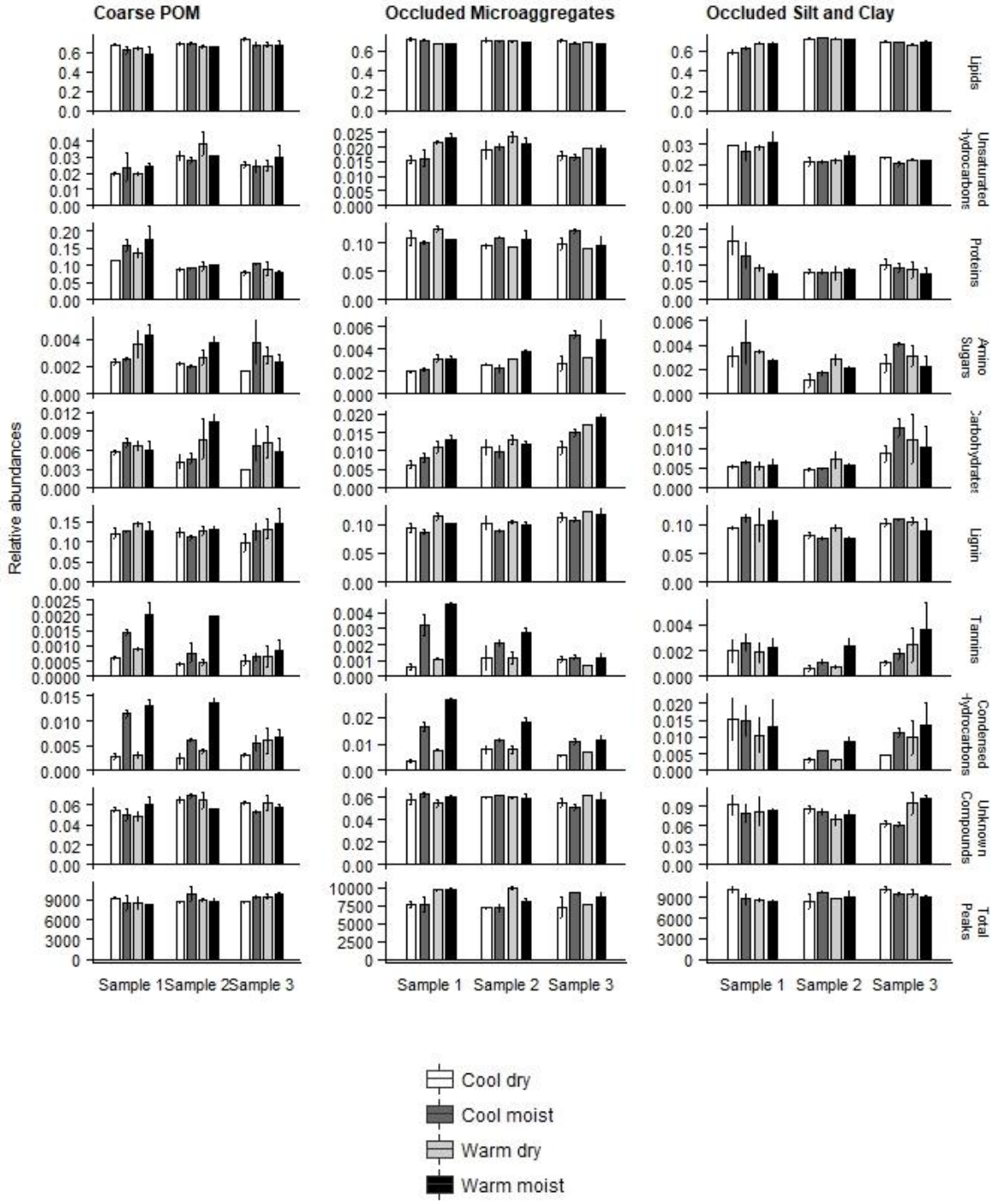


Figure 8. Proportion of microbial biomass C derived from added plant biomass, based on $\delta^{13}C$ signature. Samples 1, 2 and 3 refer to T_{30}, T_{50} and T_{60}, when ~30%, 50% and 60% of added plant biomass had been respired (see Table 1 for details), water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").

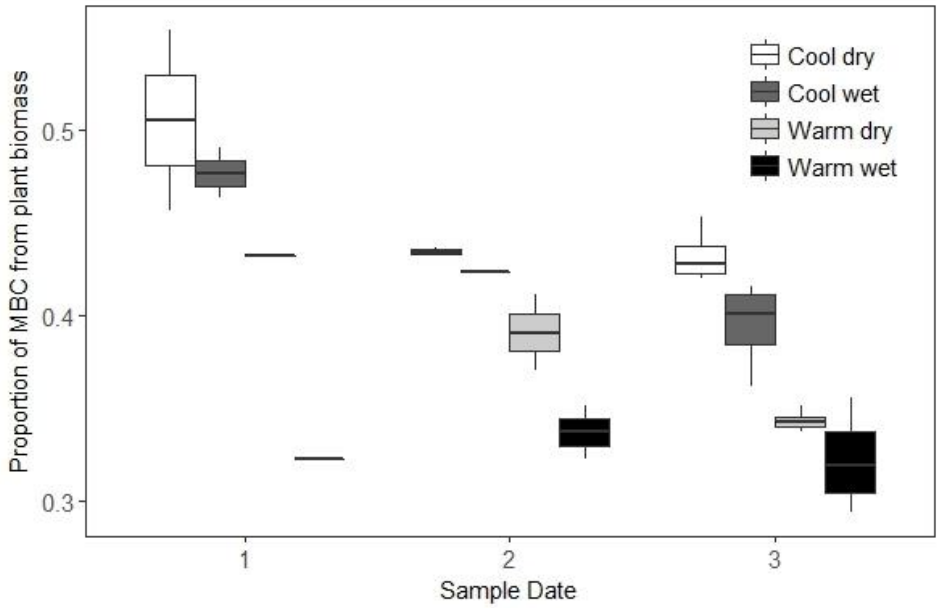


Figure 9. Efficiency of conversion of plant C to various aggregate pool C after 6 months of incubation and mineralization of ~60% of total plant C (Sample 3). Water levels were 45% or 65% WFPS (“dry” or “moist”), and temperature levels were 22 or 30°C (“cool” or “warm”).

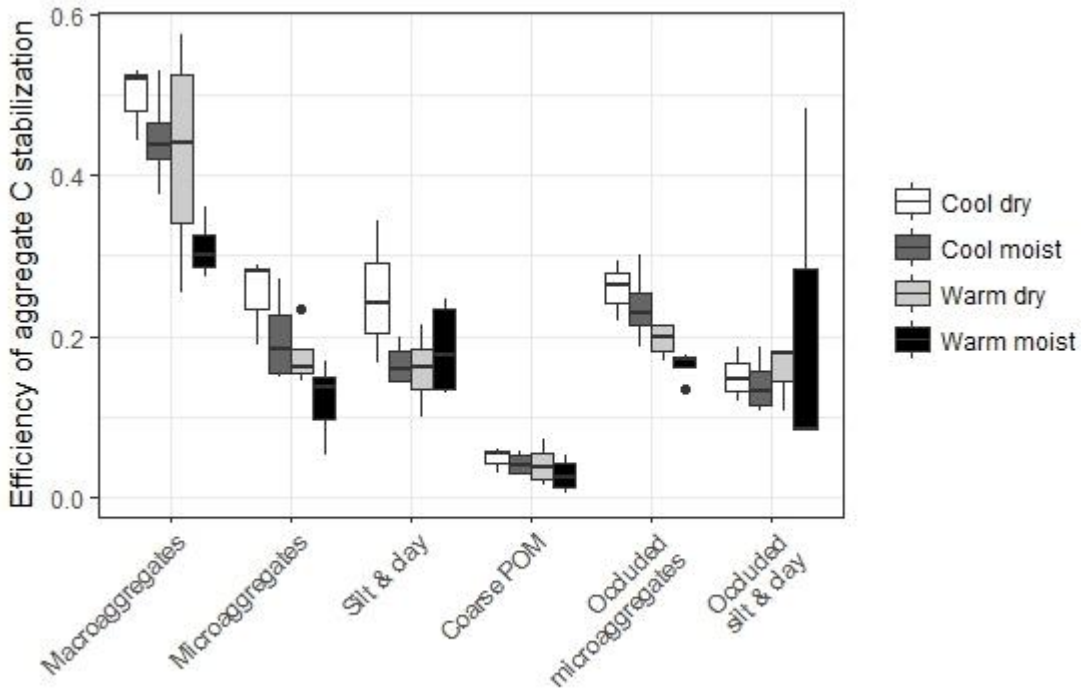


Table 1: Incubation characteristics by treatment at each sample date. *At Sample 2, control jars were not always measured on the day of sampling, so mean control values are taken from nearest measurements: Day 61 for warm dry, Day 100 for all cool. Water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").

Sample	Treatment	Time	Rate of C loss		Control rate of C loss		Cumulative CO^2 Efflux		Control Cumulative CO2 Efflux	
		days	g C day^{-1}		g C day^{-1}		g		g	
1	Warm dry	15	1.26E-02	a	2.98E-3	a	0.385	a	0.0789	a
	Warm moist	15	1.11E-02	b	2.78E-3	a	0.390	a	0.0797	a
	Cool dry	29	4.03E-03	c	7.05E-4	b	0.387	a	0.0656	b
	Cool moist	29	4.15E-03	c	7.99E-4	b	0.353	b	0.0711	b
2	Warm dry	63	2.98E-03	a	1.59E-3	a	0.665	a	0.181	a
	Warm moist	55	3.78E-03	a	1.57E-3	a	0.684	a	0.162	a
	Cool dry	99	2.52E-03	b	5.45E-4	b	0.605	b	0.115	b
	Cool moist	99	2.46E-03	b	6.64E-4	b	0.612	b	0.128	b
3	Warm dry	195	8.79E-04		3.60E-4		0.854	a	0.311	a
	Warm moist	195	1.13E-03		7.76E-4		0.922	a	0.313	a
	Cool dry	195	1.27E-03		4.45E-4		0.774	b	0.164	b
	Cool moist	195	1.17E-03		5.78e-4		0.805	b	0.188	b

Table 2: ANOVA p-values of potential activity in umol g soil^{-1} h^{-1} for the enzymes α-glucosidase, β-glucosidase, cellobiosidase, leucine-amino-peptidase, N-acetyl-glucosidase, phosphatase, and xylosidase (AG, BG, CELL, LAP, NAG, PHOS and XYL) and for microbial biomass C (MBC). Samples 1, 2, and 3 occurred when 30, 50 and 60% of biomass added had been lost, water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").

Parameter	AG	BG	CELL	PHOS	NAG	XYL*	LAP	MBC
	P-value							
Sample (S)	**<0.05**	**<0.0001**	**<0.001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.001**	**<0.0001**
Water (W)	0.64	0.68	0.68	0.38	**<0.05**	**<0.0001**	0.12	0.09
Temperature (T)	0.75	**<0.001**	0.35	**<0.001**	**<0.001**	0.19	**<0.001**	**<0.0001**
S x W	0.33	**<0.05**	**<0.05**	**<0.01**	**<0.01**	**<0.0001**	0.25	0.37
S x T	0.09	**<0.001**	0.08	**<0.001**	**<0.0001**	**<0.0001**	**<0.0001**	0.37
T x W	0.45	0.36	0.19	0.59	0.22	0.56	0.76	**<0.05**
S x T x W	0.13	0.21	0.33	**<0.05**	**<0.05**	0.59	0.47	**<0.05**

*not measured at Sample 2

Table 3: Results of PERMANOVA analysis for 16s bacterial composition and aggregate chemical composition Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry compound classes. Samples 1, 2, and 3 occurred when 30, 50 and 60% of biomass added had been lost, water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").

Parameter	16s composition	FTICR spectra					
	Whole soil	Macroaggregates	Microaggregates	Silt & Clay	Coarse POM	Occluded Microaggregates	Occluded Silt & Clay
	P-value						
Sample (S)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.01**	**<0.05**	**<0.001**
Water (W)	**<0.001**	**<0.001**	**<0.001**	0.24	.10	**<0.01**	0.29
Temperature (T)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	.07	**<0.01**	0.07
S x W	**<0.001**	0.22	**<0.05**	0.12	.47	0.16	0.93
S x T	0.06	0.12	**<0.01**	**<0.001**	.94	**<0.05**	**<0.05**
W x T	**<0.05**	0.36	0.34	0.41	.86	1.0	0.55
S x W x T	0.06	0.12	**<0.05**	0.41	.65	0.98	0.87

Table 4: ANOVA *P*-values from models of temperature and moisture effects on aggregate fraction mass, C, N, and proportion of plant-derived C in aggregate fractions. Samples 1, 2, and 3 occurred when 30, 50 and 60% of biomass added had been lost, water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").

Model Parameters	Macro-aggregates	Micro-aggregates	Silt & clay	Coarse POM	Occluded micro-aggregates	Occluded silt & clay	Whole soil
				P-value			
				Aggregate mass distribution			
Sample (S)	0.33	0.51	0.06	**<0.0001**	**<0.05**	**<0.01**	NA
Water (W)	**<0.0001**	**<0.01**	**<0.0001**	0.10	**<0.001**	**<0.01**	NA
Temperature (T)	0.64	0.54	0.82	0.50	0.22	0.29	NA
S x W	0.27	0.55	0.26	0.23	0.93	0.18	NA
S x T	0.96	0.71	0.99	**<0.01**	0.14	0.74	NA
W x T	0.36	0.22	0.81	0.67	0.45	0.90	NA
S x W x T	0.64	0.81	0.34	0.81	0.58	0.16	NA
				Aggregate C content			
Sample (S)	0.19	0.54	0.14	**<0.0001**	**<0.05**	0.87	**<0.0001**
Water (W)	0.06	0.78	**<0.0001**	0.92	**<0.05**	0.79	0.07
Temperature (T)	0.39	0.93	0.49	0.90	0.93	0.50	0.08
S x W	0.61	0.24	0.61	0.82	0.98	0.85	0.35
S x T	0.54	0.17	0.65	0.64	0.08	0.86	0.47
W x T	0.79	0.25	0.94	0.44	0.60	0.49	0.87
S x W x T	0.29	0.42	0.28	0.82	0.47	0.84	0.85
				Aggregate N content			
Sample (S)	**<0.05**	0.83	**<0.01**	**<0.0001**	**<0.01**	0.22	0.84
Water (W)	**<0.001**	0.79	**<0.0001**	0.60	**<0.01**	0.79	0.78
Temperature (T)	0.18	0.90	0.88	0.67	0.72	0.61	0.51
S x W	0.72	0.25	0.60	0.57	0.74	0.78	0.66
S x T	0.62	0.13	0.96	0.20	0.36	0.96	0.29
W x T	0.81	0.19	0.80	0.45	0.87	0.58	0.96
S x W x T	0.51	0.47	0.14	0.95	0.25	0.35	0.60

Table 5: ANOVA *P*-values from models of temperature and moisture effects on relative abundances of organic C in aggregate fractions. Compound classes were determined by Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry. Samples 1, 2, and 3 occurred when 30, 50 and 60% of biomass added had been lost, water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").

Model

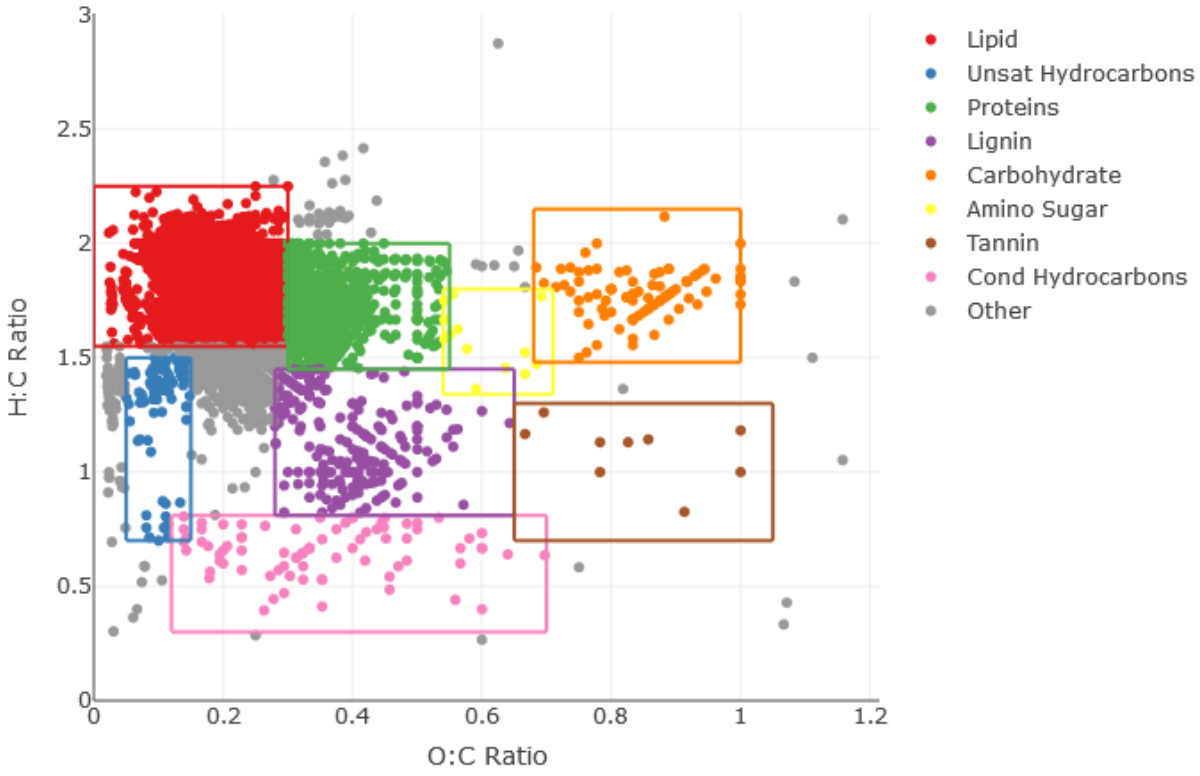
Parameters	Macro-aggregates	Micro-aggregates	Silt & clay	Coarse POM	Occluded micro-aggregates	Occluded silt & clay
	P-value					
	Total peaks					
Sample (S)	**<0.0001**	**<0.01**	**<0.0001**	0.27	0.47	0.27
Water (W)	**<0.05**	0.95	0.18	0.53	0.70	0.54
Temperature (T)	0.60	0.62	**<0.0001**	0.84	**<0.01**	0.11
S x W	0.94	0.16	**<0.001**	0.37	0.10	0.10
S x T	0.70	0.15	**<0.0001**	0.31	0.08	0.51
W x T	0.32	0.13	**<0.001**	0.53	0.23	0.99
S x W x T	0.94	0.12	0.48	0.51	0.60	0.33
	Lipids					
Sample (S)	0.11	**<0.0001**	**<0.0001**	**<0.05**	0.30	**<0.0001**
Water (W)	**<0.05**	**<0.01**	0.31	0.11	0.07	0.22
Temperature (T)	**<0.0001**	**<0.0001**	**<0.0001**	0.07	**<0.05**	**<0.05**
S x W	0.52	**<0.01**	**<0.05**	0.44	0.69	0.47
S x T	0.32	**<0.01**	**<0.0001**	0.98	**<0.05**	**<0.001**
W x T	0.86	0.31	0.57	0.65	0.68	0.66
S x W x T	0.17	0.14	0.39	0.76	0.57	0.21
	Proteins					
Sample (S)	0.16	**<0.01**	**<0.0001**	**<0.0001**	0.34	0.07
Water (W)	0.40	**<0.01**	0.99	0.05	0.38	0.31
Temperature (T)	0.71	**<0.05**	**<0.01**	0.37	0.77	**<0.05**
S x W	0.82	0.13	0.53	0.14	0.07	0.50
S x T	0.11	**<0.05**	0.26	0.42	0.13	0.08
W x T	0.23	0.44	**<0.05**	0.43	0.40	0.66
S x W x T	0.55	**<0.01**	0.71	0.61	0.82	0.91
	Lignin					
Sample (S)	**<0.01**	**<0.0001**	**<0.0001**	0.88	**<0.05**	0.05
Water (W)	**<0.01**	0.83	0.23	0.72	0.06	0.85
Temperature (T)	**<0.0001**	**<0.0001**	0.56	0.12	**<0.05**	0.90
S x W	**<0.05**	0.10	0.18	0.48	0.84	0.38
S x T	0.54	0.20	0.34	0.78	0.62	0.66
W x T	0.46	0.96	0.40	0.78	0.98	0.31
S x W x T	0.25	0.27	0.48	0.71	0.73	0.89

			Unknown Compounds			
Sample (S)	**<0.0001**	**<0.05**	**<0.0001**	**<0.05**	0.22	0.71
Water (W)	0.27	0.24	0.53	0.54	0.59	0.97
Temperature (T)	**<0.05**	0.4757	**<0.01**	0.80	0.88	0.25
S x W	0.41	0.10	0.11	0.34	0.26	0.86
S x T	**<0.0001**	0.12	**<0.01**	0.32	0.14	**<0.05**
W x T	0.15	0.72	0.32	0.56	0.94	0.38
S x W x T	0.28	0.38	0.74	0.10	0.90	0.99
			Unsaturated hydrocarbons			
Sample (S)	0.57	**<0.0001**	0.06	**<0.05**	0.10	**<0.01**
Water (W)	0.55	**<0.05**	0.14	0.86	0.82	0.92
Temperature (T)	**<0.001**	**<0.01**	0.74	0.31	**<0.01**	0.32
S x W	0.11	0.67	0.51	0.28	0.79	0.61
S x T	0.94	**<0.05**	**<0.01**	0.75	0.30	0.84
W x T	0.88	0.13	0.80	0.94	0.76	0.21
S x W x T	0.14	0.33	0.31	0.68	0.67	0.86
			Amino sugars			
Sample (S)	0.27	**<0.01**	**<0.0001**	0.41	**<0.05**	**<0.05**
Water (W)	0.42	0.67	**<0.01**	0.15	0.06	0.70
Temperature (T)	**<0.01**	0.19	**<0.0001**	**<0.05**	**<0.0001**	0.96
S x W	0.90	0.19	0.49	0.92	0.07	0.93
S x T	0.81	0.07	**<0.001**	0.21	0.38	0.22
W x T	0.52	0.25	0.23	0.74	0.99	**<0.05**
S x W x T	0.48	0.11	0.99	0.13	0.55	0.84
			Carbohydrates			
Sample (S)	**<0.05**	**<0.0001**	**<0.05**	0.63	**<0.001**	**<0.05**
Water (W)	0.05	**<0.05**	0.19	0.28	0.07	0.58
Temperature (T)	**<0.001**	**<0.001**	0.52	**<0.05**	**<0.001**	0.87
S x W	0.84	**<0.01**	0.92	0.89	0.08	0.77
S x T	**<0.05**	**<0.01**	**<0.05**	0.16	0.26	0.79
W x T	0.44	0.79	0.43	0.37	0.81	0.27
S x W x T	0.25	0.80	0.77	0.29	0.82	0.53
			Tannins			
Sample (S)	**<0.0001**	**<0.001**	**<0.0001**	**<0.01**	**<0.01**	0.20
Water (W)	**<0.0001**	**<0.0001**	**<0.001**	**<0.001**	**<0.0001**	0.11
Temperature (T)	**<0.0001**	**<0.01**	**<0.0001**	**<0.001**	0.18	0.19
S x W	**<0.0001**	**<0.001**	0.75	**<0.05**	**<0.001**	0.90
S x T	0.11	**<0.05**	**<0.0001**	0.34	0.16	0.32

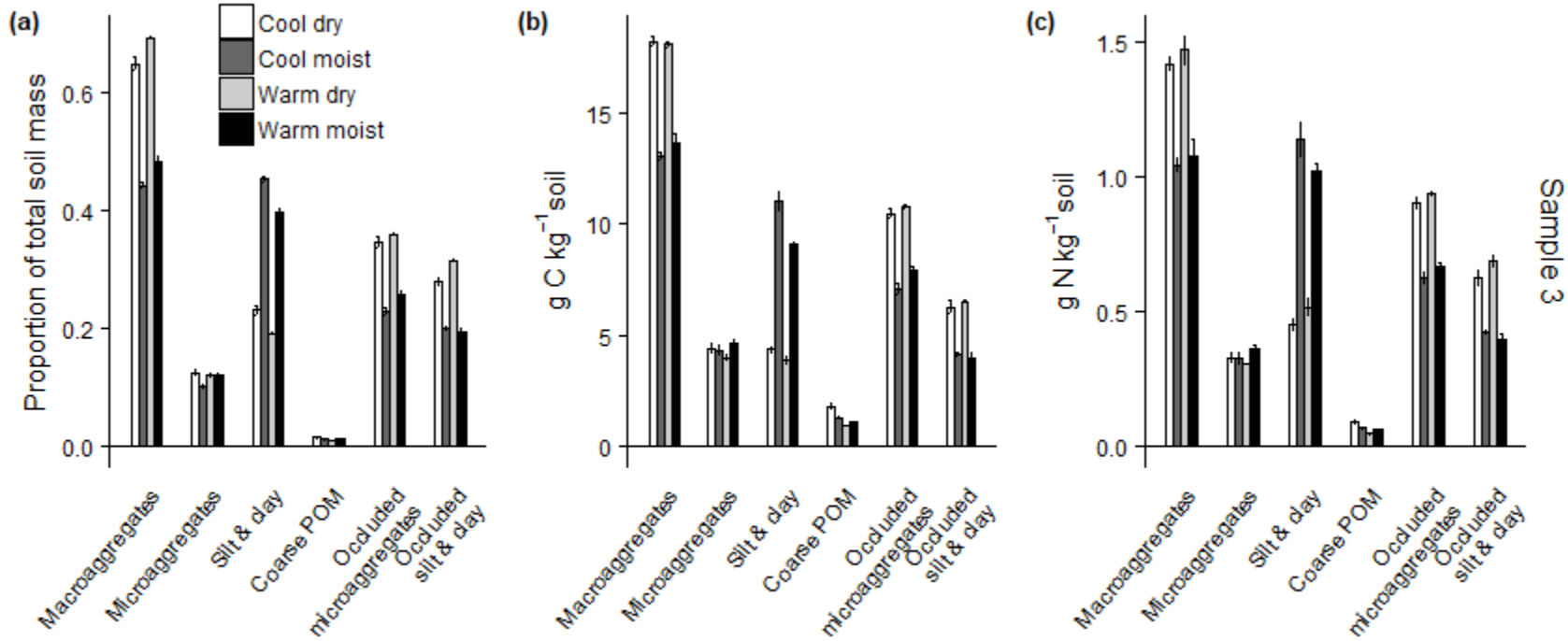
W x T	0.48	0.39	0.49	0.05	0.17	0.65
S x W x T	**<0.05**	0.06	0.25	0.20	0.92	0.87

	Condensed hydrocarbons					
Sample (S)	**<0.0001**	**<0.0001**	**<0.0001**	0.05	**<0.001**	0.06
Water (W)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	0.19
Temperature (T)	**<0.0001**	**<0.001**	**<0.0001**	**<0.01**	**<0.001**	0.81
S x W	**<0.0001**	**<0.0001**	0.46	**<0.01**	**<0.0001**	0.79
li S x T	**<0.05**	**<0.01**	**<0.0001**	0.12	**<0.01**	0.54
W x T	0.21	**<0.05**	0.06	0.18	**<0.01**	0.85
S x W x T	**<0.01**	**<0.05**	0.11	0.09	0.08	0.87

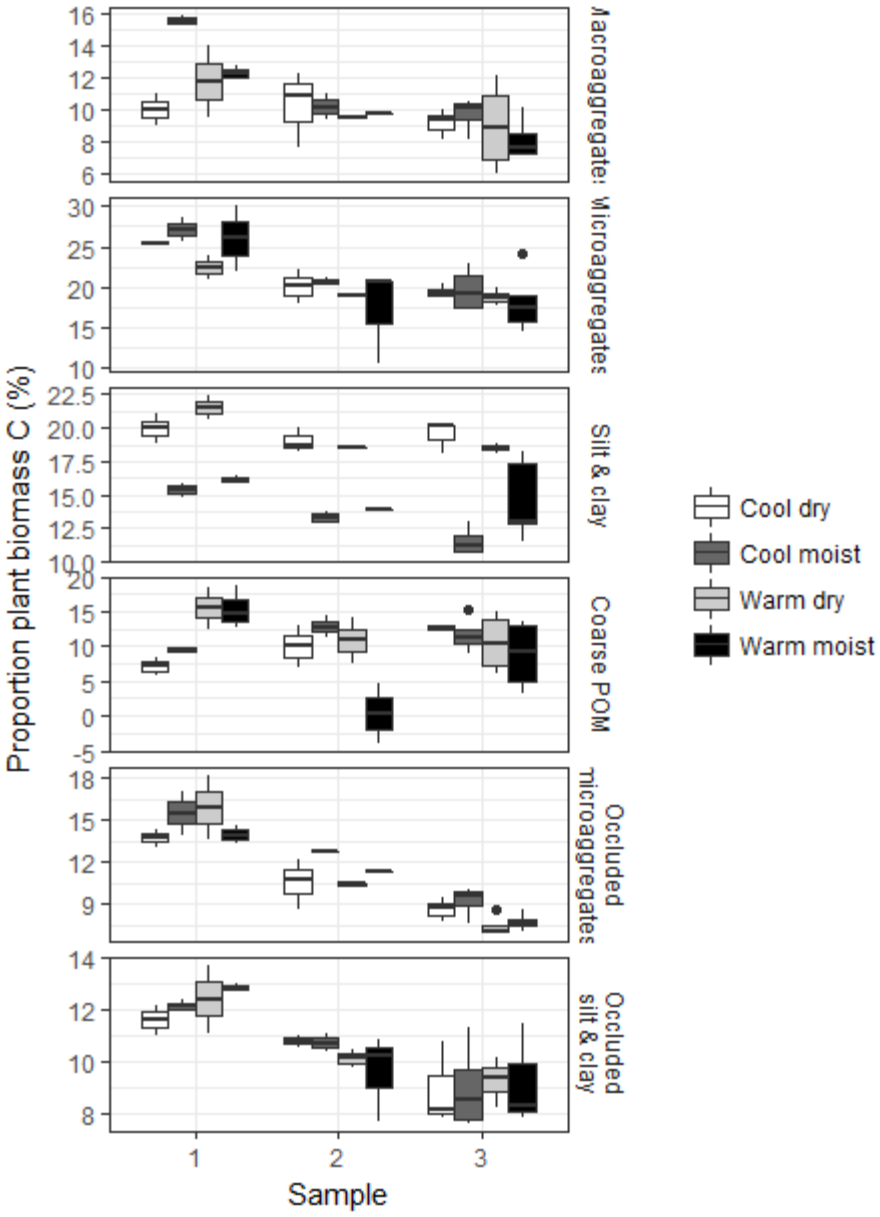
Supplemental Figure 1: Example of a van Krevlan diagram, visualizing the classification of organic C compounds according to H:C and O:C ratios. Peaks were generated using Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometry and elemental formulas assigned based on in-house software at the Environmental Molecular Sciences Laboratory.



Supplemental Figure 2. Aggregate distribution, C and N stocks in control jars (no added plant litter) after 6 months of incubation (Sample 3). Water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").



Supplemental Figure 3: Proportion of plant biomass recovered in aggregate fractions as calculated by $\delta^{13}C$. Samples 1, 2, and 3 occurred when 30, 50 and 60% of biomass added had been lost, water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").



Supplemental Table 1: ANOVA p-values of potential activity in umol g soil-1 h-1 for the enzymes α-glucosidase, β-glucosidase, cellobiosidase, leucine-amino-peptidase, N-acetyl-glucosidase, phosphatase, and xylosidase (AG, BG, CELL, LAP, NAG, PHOS and XYL) and for microbial biomass C (MBC). Samples 1, 2, and 3 occurred when 30, 50 and 60% of biomass added had been lost, water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").

	AG	BG	CELL	PHOS	NAG	XYL*	LAP	MBC
	P-value							
Model Parameters								
Control								
Temperature (T)	0.09	0.31	0.79	**<0.05**	0.85	0.19	0.15	0.27
Water	0.42	0.30	0.19	0.48	0.88	0.58	0.10	0.16
T x W	0.19	0.68	0.30	0.16	0.06	0.52	0.61	0.18
Biomass vs Control								
Addition (A)	0.52	0.87	0.65	0.16	**<0.001**	0.10	**<0.0001**	**<0.001**
Temperature (T)	0.08	0.07	0.60	**<0.05**	0.44	**<0.05**	**<0.01**	**<0.001**
Water (W)	0.72	0.41	0.67	0.32	**<0.05**	0.37	0.06	0.33
A x T	0.71	0.64	0.83	0.52	0.53	0.32	**<0.05**	**<0.05**
A x W	0.19	0.55	0.26	0.84	**<0.05**	0.12	0.47	0.10
T x W	0.32	0.77	0.50	0.42	0.10	0.56	0.76	0.13
A x T x W	0.54	0.80	0.58	0.42	0.80	0.80	0.51	0.39

Supplemental Table 2: ANOVA *P*-values from models of temperature and moisture effects on aggregate fraction mass, C and N in control jars (Control Model), ANOVA P-values of biomass addition, temperature and moisture effects on aggregate fraction C and N (Biomass vs. Control) after 6 months of incubation. Water levels were 45% or 65% WFPS ("dry" or "moist"), and temperature levels were 22 or 30°C ("cool" or "warm").

Model Parameters	Macro-aggregates	Micro-aggregates	Silt & clay	Coarse POM	Occluded micro-aggregates	Occluded silt & clay	Whole soil
	P-value						
	Aggregate mass distribution						
Control							
Water(W)	**<0.0001**	0.21	**<0.0001**	0.80	**<0.0001**	**<0.0001**	NA
Temperature (T)	**<0.05**	0.39	**<0.001**	**<0.05**	0.14	0.14	NA
W x T	0.91	0.15	0.44	0.06	0.41	0.41	NA
Biomass vs Control							
Addition (A)	**<0.0001**	0.06	**<0.0001**	0.08	**<0.0001**	**<0.0001**	NA
Water (W)	**<0.0001**	**<0.05**	**<0.0001**	0.28	**<0.0001**	**<0.0001**	NA
Temperature (T)	0.12	0.98	**<0.05**	0.32	0.14	0.90	NA
A x W	**<0.0001**	0.40	**<0.0001**	0.37	**<0.01**	**<0.001**	NA
A x T	0.13	0.20	**<0.05**	0.21	0.77	**<0.05**	NA
W x T	0.40	0.07	0.70	0.50	0.62	**<0.01**	NA
A x W x T	0.47	0.82	0.16	0.19	0.59	0.70	NA
	Aggregate C						
Control							
Water(W)	**<0.0001**	0.46	**<0.0001**	0.46	**<0.0001**	**<0.0001**	0.20
Temperature (T)	0.74	0.84	**<0.05**	**<0.05**	0.15	0.43	**<0.05**
W x T	0.53	0.35	0.18	0.17	0.58	0.23	0.36
Biomass vs Control							
Addition (A)	**<0.0001**	0.86	**<0.001**	0.10	**<0.0001**	0.07	**<0.0001**
Water (W)	0.76	0.39	**<0.0001**	0.42	**<0.0001**	0.34	**<0.05**
Temperature (T)	0.31	0.10	**<0.05**	0.06	0.80	0.45	**<0.05**
A x W	0.34	0.07	**<0.0001**	0.99	**<0.01**	0.10	0.22
A x T	0.49	0.13	0.47	0.22	0.13	0.61	**0.90**
W x T	0.36	0.54	0.71	0.56	0.74	0.91	0.64
A x W x T	0.47	0.58	0.15	0.25	0.70	0.51	0.58
	Aggregate N						
Control							
Water(W)	**<0.001**	0.43	**<0.0001**	0.39	**<0.001**	**<0.001**	0.32

Temperature (T)	0.64	0.89	0.72	**<0.05**	0.30	0.36	0.83
W x T	0.92	0.39	0.25	0.10	0.76	0.13	0.70
Biomass vs Control							
Addition (A)	**<0.0001**	0.66	**<0.01**	0.11	**<0.0001**	**<0.05**	**<0.01**
Water (W)	**<0.001**	0.53	**<0.0001**	0.35	**<0.0001**	**<0.05**	0.65
Temperature (T)	0.61	0.10	0.88	0.09	0.64	0.63	0.78
A x W	0.12	0.08	**<0.01**	0.91	**<0.01**	**<0.01**	0.39
A x T	0.26	**<0.05**	0.76	0.28	0.44	0.87	**0.97**
W x T	0.78	0.32	0.89	0.73	0.73	0.45	0.48
A x W x T	0.66	0.85	0.09	0.45	0.45	0.62	0.82

Chapter 5: Conclusions

5.1 Summary

This work was motivated by interest in the efficacy of sustainable agriculture practices regarding soil C. Since soil C processes occur on multiple scales, I performed both field and laboratory experiments and made measurable strides in understanding the effects of management and abiotic conditions on soil C.

We investigated cover crops as a tool to marginally perennialize annual cropping systems and therefore increase soil C by increasing total photosynthesis and root biomass. Cover crops were found to have a neutral effect on the overall net ecosystem C balance (NECB) in maize systems, where the residue removal or harvest was a much more important input of C to the soil. Our estimation of R_h as part of NECB revealed seasonal stimulation of heterotrophic microbes by cover crops, but no effect of cover crops on cumulative annual C losses to R_h.

Cove crops also did not alter maize residue decomposition rate, a critical management parameter for growers in northern climates and a critical input of C to the soil, as revealed by our NECB. However, cover crops increased potentially mineralizable C (PMC) and particulate organic matter (POM) C, two pools of “active” C expected to precede increases in total soil C. This finding in light of similar C balance, decomposition rate, and microbial communities with cover crops highlights that the impacts of cover crop adoption will be slow and likely limited to belowground pools, especially where biomass growth is constrained by northern climates and annual cropping systems.

Moving into the lab to investigate the effects of temperature and moisture on microbial activity and soil aggregate C, we found that warmer temperatures increased microbial activity, but drier conditions increased soil aggregation irrespective of microbial mineralization rate, biomass, or composition. Moist conditions resulted in elevated complex C in aggregates, but lower overall macroaggregate C, suggesting

that simple C compounds may be more soluble than binding when diffusiion is greater. We concluded that when agronomic management impacts moisture regime (i.e via changing ground cover), there may be previously unforseen effects on physical protection of soil C.

5.2 Future Work

Many open questions remain, but some of the most pressing include the long-term effects of cover crops on soil C and the field testing of our finding relating moisture to aggregate C.

In the first case, developing cover crop systems that maximize production of biomass is critical to fulfilling expectations that cover crops will increase soil C. This is both an agronomic and a social issue, because moving away from maize-soy rotations opens up more options for cover crop or perennial adoption, but farmers remain reliant on existing markets, crop insurance, and infrastructure that favor maize and soy. Options for incremental change include promoting cover crop adoption following corn silage and improving technology for interseeding, but to achieve meaningful increases in C inputs from cover crops will require expanding their growing season in northern climates substantially.

On the mechanistic side, it is not clear how C moves between “active” pools and long-term sequestration, so it is difficult to infer the long-term implications of the increases we observed in PMC and POM. Research investigating the turnover rate under varying management scenarios could illuminate these processes and help us to interpret our results.

Last, the phenomenon we observed in the laboratory of de-coupled microbial activity rate from soil aggregation must be rigorously tested under natural conditions. Changing moisture regimes are expected under various climate change scenarios, and our experiment did not address the impact of frequency or intensity of wetting on physical protetion of C. Microbial populations are adapted to a wide range of conditions, and it may be that specific physiological pathways we did not investigate in this study are responsible for the increased stability we observed in drier conditions.