SOIL NUTRIENT DYNAMICS AND SHIFTING MICROBIAL PROCESSES WITH INCREASING ROTATIONAL COMPLEXITY IN ROW CROPS

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Crop and Soil Sciences – Doctor of Philosophy
Ecological Food and Farming Systems – Dual Major

2017

ABSTRACT

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Agriculture faces pressure to supply increased food, fiber, and fuel, while provisioning ecosystem services. Intensive agriculture has contributed to altered global carbon (C) and nitrogen (N) cycles, and is the leading source of nitrous oxide (N_2O), a powerful greenhouse gas. New approaches are needed to improve soil nutrient management while sustaining agricultural productivity. In natural systems a strong relationship exists between aboveground diversity and belowground ecosystem controls on nutrients. While increasing the number of crops in rotation in agroecosystems can have multiple benefits, the linkages between rotational diversity, nutrient cycling pathways, and the soil ecosystem remain poorly understood. My dissertation addresses the effects of increasing crop rotational complexity on soil C and N cycles and the soil ecosystem process that regulate them. I extend this work by testing ecosystem-based measures of soil health on Michigan farms.

Long term cropping system experiments are ideal sites to examine how rotational legacy shapes belowground processes. Chapters 1, 2, and 3, focus on a cropping biodiversity experiment in place for 10 years at the initiation of my study. The gradient ranges from continuous summer annuals – corn (*Zea mays L.*) and soybean (*Glycine max.*) – to rotations that also include a winter annual (wheat; *Triticum aestivum L.*), to complex rotations with overwintering cover crops. This experiment has not received any external inputs such as fertilizer or pest control agents, so that changes across the gradient are narrowed to the effect of crop rotational complexity. In Chapter 1 I examine how labile C and N pools, soil enzyme

activities, and soil respiration respond to increasing rotational complexity. I found that pools of potentially mineralizable C were nearly twice as high on fields with a history of cover crops, compared to those without. Rotations with a legacy of cover crops sustained higher enzyme activities, significantly higher soil respiration and accumulated significantly higher total soil organic matter.

In chapter 2, I tested the relationship between increasing the number of crops in rotation and the species diversity of soil bacteria. I found no significant difference in species diversity of bacteria, but a shift in the community between rotations with and without cover crops. Taxa responsible for this shift were mainly from the *Acidobacteria* and the *Proteobacteria* which are characterized by contrasting growth and energy use strategies. I focused on denitrification in Chapter 3, a process carried out by soil microbes that produces N_2O. Rotations with cover crops had significantly higher mean N_2O flux over two growing seasons. Enzyme assays showed that denitrification was more efficient on these rotations, and rotations with cover crops also had a significantly higher proportion of genes in the N_2O-production pathway that derived from ammonia oxidizing bacteria.

Finally, in Chapter 4 I tested soil health on fields that Michigan farmers had designated as having either good or poor soil quality. Testing captured soil variability on farmer fields, but interviews with farmers revealed caveats to implementing soil health testing. My work on increasing rotational complexity revealed novel microbial controls on soil C cycling and N_2O flux, but ultimately implementing practices that enhance soil ecosystem function depends on human decisions about land use, crop production, and environmental outcomes.

Dedicated to my parents

ACKNOWLEDGEMENTS

The completion of this dissertation would not have been possible without support and guidance from an extraordinary set of mentors, colleagues, friends, and family. First and foremost as an advisor Dr. Thomas Schmidt always guided me to think critically and strive for the crux of every unique scientific problem. I am grateful for his superior insights as a microbiologist, his great appetite for learning, and for his endless patience and positive feedback. Dr. G. Philip Robertson, with generosity and wisdom, provided guidance on the smallest details of my work while shaping my development as a biogeochemist and acting as a model of consummate scientific scholarship.

I would also like to thank the other members of my committee. Dr. Stuart Grandy led the work that drew me to Michigan State University and offered incisive feedback and critical support that guided the core of my research. Dr. John Kerr constantly challenged me to think about how my research was relevant to the challenges of sustaining natural resources. Dr. Sasha Kravchenko sharpened my critical analysis, especially in the complex endeavor that is soil science, and also generously provided laboratory space for my work.

My dissertation journey spanned multiple laboratories and institutions which served to enrich my experience with many excellent lab mates and colleagues. From the Schmidt lab I thank Kwi Kim, Tracy Teal, Clegg Waldron, Heli Juottonen, Kevin Theis, John Dover, Bjorn Ostman, Keara Towery, Steve Stoddard, Ben Roller, Chris Wright, Arvind Venkataraman, Jessica Sieber, Rachel Morris, Alex Schmidt, Dishari Mukherjee, Robert Hein, and Byron Smith. From the Robertson lab and affiliates at the Kellogg Biological Station I am grateful to Bonnie McGill, Steve Hamilton, Suzanne Sippel, Sven Bohm, Dustin Kinkaid, Kate Glanville, Di Liang,

Adam Reimer, Sarah Roley, Ilya Gelfand, Mike Abraha, Leilei Ruan, and critical support from Joe Simmons, Kevin Kahmark, Neville Millar, Stacey VanderWulp, Cathy McMinn, and Jane Schuette. I was also lucky to have excellent technicians and REUs, Marcia Croft, Alessandra Zuniga, and Andrew Galimberti.

I received invaluable support from Dr. James Kells for my work in the department of Plant, Soil and Microbial Sciences. In addition, I thank Dean Bass, MSU Extension staff Paul Gross and James DeDecker, and Colleen Forestieri of the Van Buren Conservation District as well as the 13 farmers that participated in research for this dissertation. For my involvement in the KBS GK-12 program I thank Dr. Tom Getty and Dr. Sarah Bodbyl Roels, as well as Phil Robertson. I feel incredibly fortunate to have been a part of KBS, and to have interacted with all who work there, especially Dr. Julie Doll and Dr. Katherine Gross. I also owe special gratitude to several collaborators and friends: Christine Sprunger, Krista Isaacs, Steve Culman, Ariane Peralta, Marshall McDaniel, Erin Haramoto, and Carolyn Lowry.

Finally I received ceaseless support and encouragement from my parents, Mary Ellen and Kerby, my sister Elizabeth, brothers Kerby, Matthew, Sean and Julio, and from Jennifer Blesh. I love you all.

Lastly, I would like to thank my funding sources: The MSU Center for Regional Food the USDA, the LTER, the KBS GK-12 Bioenergy Sustainability Project, the USDA-SARE graduate student grant, and KBS summer fellowship.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: DISSERTATION INTRODUCTION

OVERVIEW

Agriculture faces the combined challenges of supplying food, fuel, and fiber to an increasing world population (Pachauri, 2007) while preserving environmental quality. The last half century of extraordinary growth in global agricultural production stems from improved cultivars and increased inputs, primarily fertilizer, water, and pest control agents. Nitrogen (N) fertilizer inputs alone have increased the carrying capacity of the earth by several billion people (Smil, 1997). Yet these intensive inputs to agriculture have come with widespread environmental costs. Synthetic fertilizer, frequently applied in excess, is the largest source of non-point source pollution to waterways, leading to the proliferation of 'dead zones' in coastal ecosystems (Diaz and Rosenberg, 2008). Nitrogen inputs to agriculture are also the largest source of N_2O emissions, a potent greenhouse gas and the primary degrader of stratospheric ozone (Pachauri, 2007). The widespread "leakiness" of the N cycle indicates poor nutrient use efficiency in agriculture and the breakdown of soil ecological processes known to function in systems with greater species diversity. As a result, practices that increase species diversity in cropping systems are a proposed approach to reduce chemical inputs to agriculture, enhance soil nutrient cycling processes, and reduce losses to the environment (Hooper et al., 2005).

Soil is perhaps the most complex known biological medium and contains by far the greatest species diversity on Earth (Whitman et al., 1998). Within soil, essential nutrient cycling processes occur which ultimately sustain agriculture. Soil nutrient turnover, and N cycling in particular, occur primarily via a series of microbially-mediated transformations. Understanding underlying soil ecology and key steps in nutrient transformations is critical for improving N use

in agriculture, and for promoting approaches to sustainable crop production and environmental quality.

Many of these soil processes have been altered by the displacement of biologically-based nutrient cycling with industrially-produced inputs. Before incorporation of chemical inputs to agriculture, soil fertility was sustained via plant rotation by alternating grains, legumes, and fallows. Increasing plant diversity has been shown to increase plant productivity over monocultures (Tilman et al., 2001), however this relationship may be less certain if the diversity is divided temporally and spatially with crop rotations. Plant litter is the ultimate driver of belowground nutrient and energy pathways (Hättenschwiler et al., 2005), and changes to plant litter diversity can dictate belowground ecological functions (Meier and Bowman, 2008). Still, most annual crops such as corn and soybean, occupy a field for only 3-4 months of the year. Fields in bare fallow, have no litter inputs and constantly lose organic matter via decomposition, and mineralize N, which can be leached to groundwater or denitrified, releasing N_2O in the process.

Ecological theory and evidence from natural systems indicate that greater plant diversity increases ecosystem function and soil fertility (Dybzinski et al., 2008; Tilman and Snell-Rood, 2014; Zak et al., 2003). Whether increasing spatial and temporal diversity in cropping systems through rotational complexity similarly alters soil ecosystem function is less clear. Understanding these effects could offer alternatives to agricultural systems which have fundamentally altered global ecosystems (Galloway et al., 2004; Vitousek et al., 2009) and yet are under pressure to increase production (Balmford et al., 2012).

DISSERTATION OBJECTIVES

The overarching objective of this work is to understand how increased cropping system diversity in row crops restores soil ecosystem processes, leading to sustained crop growth and improved ecosystem health. Specifically, I address the following questions in this work: How does increasing rotational complexity and plant diversity in row-crop agriculture alter soil C and N accumulation, transformation, and gaseous losses? How does rotational diversity affect the belowground microbial diversity that carries out soil nutrient cycling processes? What are the implications of increased rotational complexity for nutrient management and environmental benefits in row-crop agriculture, and what are the challenges to farmer adoption for best management practices? To address these questions my dissertation integrates methods from ecosystem ecology, soil science, molecular biology and the social sciences.

REFERENCES

REFERENCES

Balmford, A., Green, R., Phalan, B., 2012. What conservationists need to know about farming. Proc. R. Soc. B Biol. Sci. 279, 2714 –2724. doi:10.1098/rspb.2012.0515

Diaz, R.J., Rosenberg, R., 2008. Spreading Dead Zones and Consequences for Marine Ecosystems. Science 321, 926 –929.

Dybzinski, R., Fargione, J.E., Zak, D.R., Fornara, D., Tilman, D., 2008. Soil fertility increases with plant species diversity in a long-term biodiversity experiment. Oecologia 158, 85–93. doi:10.1007/s00442-008-1123-x

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vöosmarty, C.J., 2004. Nitrogen Cycles: Past, Present, and Future. Biogeochemistry 70, 153–226–226.

Hättenschwiler, S., Tiunov, A.V., Scheu, S., 2005. Biodiversity and Litter Decomposition in Terrestrial Ecosystems. Annu. Rev. Ecol. Evol. Syst. 36, 191–218. doi:10.1146/annurev.ecolsys.36.112904.151932

Meier, C.L., Bowman, W.D., 2008. Links between plant litter chemistry, species diversity, and below-ground ecosystem function. Proc. Natl. Acad. Sci. 105, 19780–19785. doi:10.1073/pnas.0805600105

Pachauri, R.K., 2007. Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC.

Smil, V., 1997. Global population and the nitrogen cycle. Sci. Am. 277, 76–81.

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T., Lehman, C., 2001. Diversity and Productivity in a Long-Term Grassland Experiment. Science 294, 843 –845. doi:10.1126/science.1060391

Tilman, D., Snell-Rood, E.C., 2014. Ecology: Diversity breeds complementarity. Nature 515, 44–45. doi:10.1038/nature13929

Vitousek, P.M., Naylor, R., Crews, T., David, M.B., Drinkwater, L.E., Holland, E., Johnes, P.J., Katzenberger, J., Martinelli, L.A., Matson, P.A., Nziguheba, G., Ojima, D., Palm, C.A., Robertson, G.P., Sanchez, P.A., Townsend, A.R., Zhang, F.S., 2009. Nutrient Imbalances in Agricultural Development. Science 324, 1519 –1520.

Whitman, W.B., Coleman, D.C., Wiebe, W.J., 1998. Prokaryotes: The unseen majority. Proc. Natl. Acad. Sci. 95, 6578 –6583.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant Diversity, Soil Microbial Communities, and Ecosystem Function: Are There Any Links? Ecology 84, 2042–2050. doi:10.1890/02-0433

CHAPTER 2: CROP ROTATIONAL COMPLEXITY SHIFTS MICROBIAL PROCESSING AND STORAGE OF SOIL ORGANIC CARBON

ABSTRACT

Increasing rotational complexity in agroecosystems can have multiple benefits, from reducing disease pressure to increasing crop yield. Increased rotational complexity can also alter soil nutrient cycling, but linkages between rotational diversity, altered nutrient cycling pathways, and the microbial communities that carry out these processes remain poorly understood. I tracked multiple pools and fluxes of carbon (C) and nitrogen (N) along a gradient of increasing cropping system complexity from continuous corn or soybean monoculture to up to five plant species in 3-year rotations – including winter cover crops – in treatments that have not received external inputs. Under fields with different rotational histories, but under the same crop, I measured labile C pools, soil enzyme activities, mineralization potentials, and soil respiration over two growing seasons. I found that the history of crop rotation influenced soil organic carbon (SOC), which was higher under cover cropped treatments. In addition, the manner in which soil C was processed also differed by rotation. Over two seasons, rotations with cover crops respired at least 20% more CO_2 but total SOC did not reflect estimated C returns to soil from crop growth. Over the growing season, treatments with cover crops supported greater labile C pools, potential mineralization rates and patterns in microbial enzyme potentials. Results from this research show the strong effect of increasing rotational complexity on C and N pools and fluxes, as well as an unexpectedly clear and consistent reshaping of the microbial processes that underlie different crop rotations.

INTRODUCTION

To meet ever-increasing pressure to supply food, fiber and fuel, row crop agriculture has achieved higher yields through a combination of improved crop genetics and agronomic practices. In the U.S. Midwest, this has resulted in intensified grain production primarily in monocultures, with plant diversity largely limited to annuals grown in short rotations (e.g. continuous corn or soybeans) (Broussard and Turner, 2009; Klopatek et al., 1979). The management inputs needed to maintain these cropping systems have substantial environmental costs, including soil erosion, pollution of ground and surface waters, and release of greenhouse gases including nitrous oxide (IPCC, 2013). Nitrogen (N) pollution is a particularly important concern in the U.S. Midwest (Robertson and Vitousek, 2009), where N is the nutrient that most frequently limits crop yields. The Corn Belt region of the upper Midwest contributes the majority of the nitrate pollution causing a hypoxic zone to form in the Gulf of Mexico (David et al., 2010; Rabalais et al., 2002), and the large N fertilizer inputs also contribute to greenhouse gas emissions (Shcherbak et al., 2014).

A proposed method to sustain productivity while reducing N fertilizer and other agronomic inputs and environmental costs is to increase cropping system diversity to enhance ecosystem functions (McDaniel et al., 2013; Shennan, 2008). A strong body of evidence shows a relationship between biological diversity and multiple ecosystem functions such as energy flow, nutrient cycling, pest and disease suppression, and net primary productivity, due to a range of biotic interactions (Hooper et al., 2005). Soil nutrient cycling, for example, encompasses the breakdown and release of matter and energy, which is largely a function of microbial activity (Wardle, 2002), as well as the uptake and recycling of nutrients. These nutrient cycling processes are more efficient in ecosystems with diverse plant communities such as grasslands

and forests due to the broad array of ecological strategies by which species compete for and capture scarce resources (Smith et al., 2013; Tilman et al., 2001).

By contrast, agricultural systems with low plant diversity have reduced capacity to either capture or retain nutrients while also undergoing frequent disturbances that further disrupt nutrient cycles. Indeed, an increased dependence on externally supplied nutrients in agroecosystems with reduced capacity to cycle and retain nutrients results in inefficient, 'leaky' systems (Liebman et al., 2013). Soil organic carbon (SOC) is the pivotal resource for maintaining and supplying crop nutrients (Robertson and Grandy, 2006). Even in temperate cropping systems with high inputs of industrial fertilizer, grain crops rely on decomposition of SOC for approximately half of their aboveground N (Gardner and Drinkwater, 2009). Since SOC ultimately derives from plants, cropping system diversity strongly influences the quantity and quality of this input. Low-diversity agricultural systems can have highly degraded SOC stocks, and, as shown at cropping system research sites, net C inputs can vary by an order of magnitude due to management practices such as plant species selected for crop rotations, harvest and tillage practices, or fertilization (Robertson and Grandy, 2006; Sanford et al., 2012).

The composition of SOC ranges from labile pools of fresh and partially decomposed plant litter to active microbial biomass and dissolved organic matter, to more stable pools of physically protected, mineral-bound, and chemically recalcitrant SOC which can remain in soil from years to decades and longer (Schmidt et al., 2011). The energy in SOC substrates is the key source of most microbial growth and metabolism. Soil microbes access SOC resources by releasing extracellular enzymes which reflect a diverse spectrum of biochemical metabolism of labile, plant polymers (e.g. carbohydrates and proteins) via hydrolytic enzymes, and complex, humic and colloidal substances via oxidative enzymes (Sinsabaugh et al., 2009). As microbes

access energy from SOC, they release nutrients (e.g. N and phosphorus (P)) in plant available forms. The sustained supply of mineralized nutrients from SOC directly influences plant productivity (Andrén et al., 1995; Borer et al., 2005) and over time, the activity of the soil microbial community drives the fate of SOC pools: from respiration rates to eventual depletion or accrual.

Mineralization processes also inform contemporary understanding of how soils accumulate and store soil C over longer time spans (Schmidt et al., 2011), with the microbial community acting as a conduit for flows of C and N from plant litter into stabilized SOC (Grandy and Neff, 2008). Long-term stabilization of C in soil occurs by its complexation with the surfaces of clay and silt particles. Rather than this stable C deriving from recalcitrant plant C, an emergent hypothesis suggests that higher quality plant litter, which soil microbes can process more efficiently (i.e., less is respired as CO_2), is a dominant source of microbial byproducts which complex with mineral soil (Cotrufo et al., 2013).

Cropping practices which can increase the timeframe of primary productivity and supply more diverse root and plant residue inputs to soil may increase microbial activity and associated processes that stabilize C in soil (Gregorich et al., 2001; Kallenbach et al., 2015; 2016; Liang and Balser, 2011). Adding rotational complexity to grain-based agroecosystems by cultivating a greater number of plant functional types such as legumes, perennial forages, or overwintering cover crops may maintain the SOC reservoirs that mineralize and supply nutrients to the ensuing crop. Further, comparisons in long-term cropping systems have demonstrated that ecological processes can replace synthetic inputs in grain cropping systems to sustain productivity while reducing environmental impacts (Clark et al., 1998; Drinkwater et al., 1998; Gregorich et al., 2001; Robertson et al., 2014) For example, Smith et al. (2008) showed a pattern of higher crop

yields with increasing crop diversity in the absence of synthetic inputs, concluding that increased rotational complexity can maintain productivity. The results suggested that enhanced soil nutrient cycling under more diverse crop rotations increased crop yield, however the mechanisms leading to this pattern are unclear. Since row crop systems have less aboveground biodiversity than natural ecosystems, changes to crop species composing a rotation (separated temporally), as well as the degree of spatial or temporal overlap (e.g., inclusion of overwintering crops), may lead to large changes in ecosystem functions – such as soil C storage, crop productivity, and nutrient retention (Shennan, 2008; Tiemann et al., 2015; Wood et al., 2016).

Here we investigate how increasing crop rotational complexity impacts microbial enzyme activity and SOC oxidation. In a long term cropping system study we measured belowground nutrient cycling processes for two full growing seasons along a rotational complexity gradient. The gradient ranges from simple rotations of continuous summer annuals – corn (*Zea mays L.*) and soybean (*Glycine max.*) – to rotations that also include a winter annual (wheat; *Triticum aestivum L.*), to more complex rotations that include overwintering cover crops (i.e., non-harvested legumes and grasses). In this experiment, the only factor that differs among the treatments is crop rotation—tillage practices are the same, and there are no synthetic inputs to the systems. I sampled along a range of simple to complex rotations that had been in place for ten years at initial sampling. In order to test hypotheses about how crop rotational complexity alters SOC dynamics over time, I measured labile organic matter pools; the activity of microbially-produced enzymes that break down organic matter; and respired CO_2.

Specifically, we hypothesize that SOC will increase in crop rotations with greater plant C inputs to soil, larger labile soil C pools, and altered processing of SOC by soil microbes. We expect that the activities for hydrolytic soil enzymes will increase in proportion to the size of

labile C pools. By contrast, in cropping systems that result in smaller labile C pools, we expect higher activities of oxidative enzymes, which break down more recalcitrant soil C substrates. Further, we predict that larger pools of labile C will occur under complex crop rotations leading to higher soil respiration rates and but also gains in SOC, compared to reduced soil respiration and reduced SOC in simpler rotations. In this experiment, total C inputs are greater in the more complex rotations and they are also more continuous (i.e., the cover cropped treatments have year-round plant cover). We predict that the more frequent plant C inputs under more complex rotations will sustain larger labile C pools, and result in distinct microbial C processing patterns and greater mineralization potentials throughout peak crop growth periods.

METHODS

Site descriptions and experimental layout

We conducted our study at Michigan State University's Kellogg Biological Station (KBS) Long Term Ecological Research experiment located in Hickory Corners, Michigan (42° 24′ N, 85° 24′ W, elevation 288 m). KBS lies in SW Michigan, in the eastern part of the U.S. Corn Belt. Prior to 1988, the site had been conventionally managed for row crop agriculture (Robertson et al., 1997). Soils developed on glacial outwash are composed of Kalamazoo (Fine-loamy) and Oshtemo (Coarse-loamy) mixed, mesic, Typic Hapludalfs (FAO soil order: Luvisols). The climate is temperate, with approximately 1005 mm rainfall and an average snowfall of ~1.3 m and mean annual temperature is 10.1° C (Robertson and Hamilton, 2015).

This work was undertaken on the KBS Biodiversity Gradient Experiment (http://lter.kbs.msu.edu/research/long-termexperiments/biodiversity-gradient/) which consists of a gradient of rotational complexity of harvested annual grains including corn, soybean, and

wheat ranging from continuous monoculture (e.g., continuous corn, or continuous soybean) to various rotations that also include a non-harvested overwintering cover crop of red clover (*Trifolium prantense L.*), crimson clover (*Trifolium incarnatum*), or annual ryegrass (*Lolium multiflorum Lam.*) either alone or in combinations. The study was established in 2000 and is composed of plant species treatments from bare ground, to 1, 2, 3,4, 6 and 10 species, in a randomized complete block experiment with four replicates within 9 x 30 m treatment plots and with every phase of each crop rotation represented every year. Since establishment, none of the treatments have received synthetic chemical inputs (i.e. no N fertilizer or pest control agents) and weeds are controlled mechanically.

Sample collection

For this study we selected seven treatments (Table. 2.1): continuous corn (cC) and continuous soy (cS), corn and soybean in rotation (CS), CS and wheat in rotation (CSW), CSW with inclusion of clover varieties as a winter cover crop (CSW1cov), CSW with clovers and annual ryegrass (CSW2cov), and a fallow plot (Fspring) tilled once each spring to stimulate a diverse, early successional vegetation. We followed these same treatments through two growing seasons, first in 2010 with all treatments in the corn phase of the rotation (except for cS and Fspring), and again in 2011, with all treatments planted to soybeans (except for cC and Fspring). Each year sampling began at planting, in June, and continued over four months and included all treatment blocks. Within a central row of each plot soil cores were taken to a 10 cm depth from five locations beginning from 6m to 18m from the start of a row, alternating cores from within and between crop rows. Cores were composited by plot and placed on ice for transport to the lab. Within 24 hours of collection soils were sieved (to < 4mm), mixed, and weighed for soil

moisture, enzymes and mineral N analyses. A portion of the soil was air-dried for approximately 30 days.

Soil carbon and nitrogen

A portion of air-dried soils was finely ground to determine total SOC and total soil organic nitrogen (SON) by dry combustion on a Costech ECS 4010 CHNSO Analyzer (Costech Analytical Technologies, Valencia, CA). Potentially mineralizable carbon (PMC) was adapted from Franzluebbers et al. (2000) and Haney (2008) using air-dried soil brought to 50% water-filled pore space (WFPS). Deionized water was added to 15 g of air-dried soil to 50% water-filled pore space in canning jars with lids fitted with airtight, rubber septa. Lids were placed loosely on the jars and soils were incubated in the dark at 25° C. At 24-hours, jars were removed and soil respiration determined using a LI-COR LI-820 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE): jars were sealed and 0.5 mL of headspace gas was immediately removed and injected into the gas analyzer, followed by three additional measurements over approximately 90 min. PMC was calculated as CO_2 flux per gram of incubated soil.

Soil mineral N was extracted from fresh soil within 24 hours of sampling. Triplicate 10.0 g subsamples of sieved soil were extracted with 1M KCl for one hour on a rotary shaker (120 rpm). Samples were filtered and stored at -20 °C until analysis for ammonium (NH_4^+) and nitrate (NO_3^-). Potentially mineralizable nitrogen (PMN) was assessed in a short term anaerobic incubation according to Drinkwater et al. (1996). Briefly, 10 mL deionized water was added to triplicate 8 g fresh, sieved soil in conical tubes. Dinitrogen gas was used to replace tube headspace air and bubbled into the slurry for one minute prior to sealing with butyl rubber stoppers. Sealed tubes were incubated at 25 °C for seven days. After seven days the stoppers

were removed, buffer was added to bring the slurry to 1M KCl, and samples were shaken, filtered, and stored in the same manner as fresh samples. Concentrations of NH_4^+ and NO_3^- were determined colorimetrically on a 96-well plate-reader. Soil NO_3^- was determined according to Doane and Horwáth (2003) and read at 540 nm, and soil NH_4^+ concentration determined at 630 nm (Sinsabaugh et al., 2000). After incubation and extraction, PMN was determined based on the concentration of NH_4^+ incubated soil minus NH_4^+ from initial soil extraction of the same soil.

Soil Enzymes

We measured soil enzyme potentials from slurries of fresh soils following established methods (German et al., 2011; Sinsabaugh et al., 2002). First, soil pH was determined using 10 g fresh soil mixed with 20 mL of deionized water, which was allowed to equilibrate for one hour with occasional stirring. Following this determination, the pH of a 50 mM sodium acetate buffer was adjusted to the pH of treatment soils. Approximately half of 100 mL of sodium acetate buffer was added to 1 g of treatment soil, and homogenized vigorously in a blender for exactly one minute. The rest of the buffer was used to rinse remaining soil contents from the blender into the slurry and then stored at 4° C until analysis (within 1-2 hours). We evaluated the breakdown of organic compounds in soil slurries by measuring enzymatic potentials for four hydrolytic enzymes, β-1-4-glucosidase (BG), β-1-4-N-acetyl-glucosaminidase (NAG), acid phosphatase (PHOS), and tyrosine amino peptidase (TAP), and for two oxidative enzymes - phenol oxidase (PHENOX) and peroxidase (PEROX). At initiation of enzyme assays, soil slurries were constantly stirred and the suspension was pipetted into 96-well plates. Hydrolytic enzyme activity was monitored using substrate specific polymers containing fluorescent labels (either 4-methylumbelliferone or methylcoumarin). Phenol oxidase and peroxidase activity were assessed by monitoring oxidation of L-DOPA, with 0.3% hydrogen peroxide added to the latter

assay. For all enzyme assays, the exact time was noted when substrate was added to the buffered soil slurry. All enzymes were incubated in the dark at 15° C. At 4-6 hours, hydrolytic enzyme reactions were stopped by adding 10 µL of 1M sodium hydroxide, and the exact time was noted and plates were read on a Fisher Scientific Thermo Fluoroskan Ascent^{TM} microplate fluorometer (Waltham, MA). Between 6-8 hours, color change of oxidized L-DOPA was assessed colorimetrically at 450nm. Background absorbance-emission or fluorescence was accounted for with controls for buffer, buffer + soil, buffer + substrate.

In situ *soil respiration*

At approximately 2-week intervals soil CO_2 fluxes were measured using static 25 cm diameter chambers placed *in situ* in each treatment. Using a method previously described by Hoben *et al*. (2011), PVC chambers were inserted in the field mid-way between rows and central furrows to a depth of 8 cm. The soil surface inside the chamber was maintained free of living plant material. At each sampling time, chamber height from soil surface was measured to account for headspace volume and then chambers were sealed with an air-tight, O-ring PVC lid, fitted with a Vacutainer serum vial septa (Becton-Dickinson, East Rutherford, NJ). Using a syringe, and mixing the headspace at each sampling, 10 mL gas samples were removed via the lid septa, at time zero and three additional 20-minute intervals, and stored in 5.9 mL gas Exetainers (Labco, Ceredigion United Kingdom). Samples were analyzed using gas chromatography (Hewlett Packard 5890 Series II, Rolling Meadows, IL, USA) with gases separated on a Poropak Q column (1.8 m, 80/100 mesh) at 80 °C. CO_2 was analyzed using an infrared gas absorption analyzer (LI-820 CO_2 analyzer; LI-COR, Lincoln, NE, USA) and N_2O was analyzed with a ^{63}Ni electron capture detector at 350 °C.

Statistical approach

Data for all soil analyses were examined for normality and heterogeneity of variance assumptions following Zuur et al. (2010). Data deviating from normality were log-transformed prior to analysis, and homogeneity of variance checked with Levine's test. Analysis of variance was calculated in R (The R Foundation for Statistical Consulting, Vienna, Austria) using the *lme4* package for linear, mixed-effect (Bates et al., 2016), with sampling time and block as random effects and crop rotation as a fixed effect. Results are reported as statistically significant at $\alpha = 0.05$.

RESULTS

Soil Carbon and Nitrogen

Across the rotation, SOC differed significantly by rotation ($P < 0.019$), (Table 2.2), with comparisons showing cC to be the most deficient in SOC when compared to CSW1cov ($P < 0.0703$) and to CSW2cov ($P < 0.0510$). The more simplified rotations (cS, CS, CSW) were all significantly lower than rotations with cover crops, with mean SOC ranging between 7.93 and 7.95 g-C kg $^{-1}$ soil (Table 2.2). For SON, the effect of rotation was not significant. Among treatments, comparisons showed a similar trend of cC's having the smallest SON pool and cover-cropped rotations the highest values (Table 2.2). Soil C:N was not different across rotations, nor were there any clear trends by rotation, except for cS tending to have a lower C:N than all other rotations (Table. 2.2).

Mineralization potentials for C followed similar patterns across treatments for both corn and soybean years,; they were significantly higher in cover cropped treatments than in no-cover treatments (Fig. 2.1, Table 2.4). PMC was higher in the corn year for all rotations compared to

the soybean year, but the magnitude of the difference between cover and non-cover was larger in the corn year than in the soybean year. The magnitude of PMC under corn shifted dramatically over the season, more than doubling between June and July for all treatments except in cC (Fig. 2.1). PMC was higher for CS and CSW than in cC in July and August. In both corn and soybean years, the different cover crop treatments had nearly identical PMC over the course of the season, but in the soybean year, the July increase in PMC was not as high as under corn, and the overall mean PMC was lower in the soybean year (Table 2.4).

Soil Enzyme Activity

In both 2010 and 2011, beta-glucosidase (BG) activity was consistently higher under cover cropped treatments (Table 2.3), and activities for all treatments spiked and fell off during the growing season, leveling off between August and September with activities considerably lower than in 2010 (Fig. 2.2). The pattern for the N-acquiring enzymes, N-acetyl-glucosaminidase and tyrosine amino peptidase (NAG+TAP), was clearly different in cover vs. no-cover treatments (Fig. 2.2). In both years, for nearly all crop rotations, these activities spiked in July and then fell. For acid phosphatase (PHOS), activities differed in both magnitude and pattern between years (Fig. 2.2). In 2010, PHOS activities increased dramatically between June and August, doubling for no-cover treatments, and nearly tripling for cover cropped treatments, before falling in September. In 2011, PHOS activities were generally steadier and fell slightly over the growing season. PHOS activities under cover cropped treatments were again significantly higher than for no-cover rotations, except for CSW in 2011 (Table 2.3).

The activities of the oxidative enzymes peroxidase and phenol oxidase (PEROX and PHENOX), contrasted with the hydrolytic enzymes with increasing rotational complexity. For

both corn and soybean years, rotations with winter cover tended to have similar or lower PEROX + PHENOX enzyme activities compared with the no-cover treatments (Fig. 2.2). In the corn year of the study, the oxidative enzymes spiked early and then fell: in July the highest enzyme activity occurred in cC, whereas CSW1cov had the lowest activity of the oxidative enzymes. However, for all other months during the corn year, the difference in oxidative enzyme activity by rotation was nearly indistinguishable. In the soybean year, the highest oxidative enzyme activity tended to occur in cS, except for in August, when there was higher oxidative activity under CS and CSW. These two rotations had the greatest fluctuation in oxidative enzyme activities over both growing seasons. In contrast, the cover crop treatments tended to be less variable and were consistently lower than continuous cS, and in no month did the oxidative enzyme activity in either cover crop treatment exceed the highest activities in any of the no-cover treatments (Fig. 2.2). The magnitude of oxidative enzyme activities was much greater in the soybean year than in the corn year, and patterns in activity differed between PHENOX and PEROX (Table 2.3). The pronounced spikes and month-to-month variability in both years make rotational-level differences less clear overall. However, the highest oxidative enzyme activities tended to occur in cC and cS, and peroxidase activities were also generally higher in less-diverse rotations than they were in the more complex rotations with cover crops.

The mean ratio of hydrolytic enzymes to PMC did not differ for any potential activities for either year, and no clear trends emerged in this metric based on crop rotation history (supplemental Fig. 2.1). In contrast, the ratio of oxidative enzymes to PMC (OX:PMC, Table 2.4) was strongly significant by rotation for both 2010 ($P < 0.0001$) and 2011 ($P < 0.0001$). In 2010, this ratio was significantly lower for the cover-cropped rotations. A similar pattern was

observed in 2011, with the highest OX:PMC ratios in continuous monocultures, followed by CS and CSW, and the lowest ratios in the cover cropped treatments (Fig. 2.3).

The ratio of hydrolytic enzymes to oxidative enzymes was strongly dependent on crop rotation history. The ratio of BG, NAG+TAP and PHOS to OX was higher in rotations with cover crops for both years (Table 2.4, supplemental Fig. 2.2). The relationship was strongest for N-acquiring enzymes ([NAG+TAP]:OX), with treatments with winter cover crops having a significantly higher ratio under both corn ($P<0.001$) and soybean ($P<0.0023$). The ratio of BG:OX was strongly significant by rotation in the corn year ($P<0.0038$), and was less strong in the soybean year, although there was a similar pattern by rotation. Ratios for PHOS:OX also tended to be higher under rotations with winter cover, and were marginally significant in both 2010 ($P<0.0453$) and 2011 ($P<0.0847$). These trends are even more notable given the fluctuation in different enzyme activities over the course of both growing seasons.

In situ *soil respiration*

Clear patterns emerged in soil respiration by rotational history. In 2010, *in situ* soil CO_2 flux was similar for all of the rotations that included corn (Table 2.5). Indeed, through most of the season CO_2 fluxes were not significantly different across treatments at most time points – even when including cS, which had significantly lower soil respiration rates than did rotations with corn, (Fig. 2.4). In 2010, only the first and last fluxes differed across all crop rotations (Fig. 2.4), though over the season the highest fluxes were observed in the most diverse rotations (CSW2cov) and the lowest fluxes in the rotations with lowest diversity (cC and cS; supplemental Table 2.2). In 2011, soil respiration in the cover cropped treatments was even more distinguishable from no-cover rotations, with time points earliest in the season leading to the

greatest divergence in flux measures between rotations (Fig 2.4, supplemental Table 2.2). Overall, mean soil respiration rates were much lower in 2011 than in 2010, but the significantly higher respiration in cover-cropped treatments was more apparent in 2011 (Table 2.5).

Labile nitrogen

In 2010, inorganic N as both NH_4^+-N and NO_3^--N was significantly higher in the CSW1cov rotation, containing only a legume cover (supplemental, Table 2.1). Beginning from a high concentration, inorganic N in CSW1cov generally remained higher than in all other treatments over the course of the growing season, until September when it fell below CSW2cov (supplemental Fig. 2.3). NH_4^+-N was significantly higher in CSW2cov than in the less diverse rotations, cC and CS, but not as high as it was in CSW (supplemental, Table 2.1). CSW2cov had a more pronounced drop and recovery in NH_4^+-N concentration over the growing season compared to NO_3^--N, which retained a steadier concentration over time. Of the rotations without cover crops, CS generally had the least available N as either NH_4^+-N or NO_3^--N, and CSW generally had higher NH_4^+-N over the season (supplemental Fig. 2.3). In the rotations without cover crops, cC had intermediate levels of labile N species over the growing season. In both years, NH_4^+-N concentrations varied less throughout the season than the cover cropped treatments. The CSW2cov treatment barely fluctuated around a mean of 2 μg NH_4^+-N per gram of soil over the season, CSW1cov was generally higher in NH_4^+-N, and fluctuated a bit more, and the greatest variations in NH_4^+-N across the season were in the cS and CS rotations (supplemental Fig. 2.3). For NO_3^--N, the CSW1cov treatment generally had far higher concentrations over the season except in August when it fell dramatically, and then recovered in September. The CSW2cov treatment showed a similar drop followed by an increase, but

generally followed a closer pattern to the less complex treatments in NO_3^--N across the season. In both years of the study, PMN was not different among no-cover rotations, but significantly higher by a factor of ~2 in treatments with cover crops (supplemental Table 2.1).

DISCUSSION

Introducing more plant diversity into grain crop rotations can improve crop productivity and agronomic performance, but the soil ecosystem processes underlying these observations are poorly understood. I examined how increased crop rotational complexity alters mechanisms that control microbial metabolism and relative SOC pool size. Results support the hypothesis that SOC pools increase, and soil C metabolism changes, with increasing rotational complexity, but almost all differences were due to the presence of a cover crop in rotation rather than crops in rotation *per se*. I found that during both corn and soybean growing seasons PMC and hydrolytic enzyme activities were significantly higher in crop rotations with cover crops than in rotations without cover. By contrast, no-cover treatments had higher oxidative enzyme activities, especially relative to PMC. This pattern in enzyme potentials suggests that the microbes in cover crop treatments are processing more labile C. Even though total plant residue returns, normalized across all multi-year rotation types, differed little for some of the rotations with and without cover (supplemental Fig. 2.4), complex rotations with cover crops respired more CO_2 over the growing season and have accumulated more SOC over time. The quality and/or frequency of C inputs from overwintering cover crops appear to be the critical factors altering microbial metabolism, soil respiration, and net C accrual.

In this long term experiment, inputs from an overwintering cover crop shaped patterns in litter decomposition (flows, rates, turnover), more than the current or previous grain crop in rotation. In the corn year of this study, CS had been in soybean, CSW, CSW1cov and CSW2cov had been in wheat, and in the soybean year, all rotations, except cS, had previously been in corn (Table 2.1). While Smith et al. (2008) presented data from this experiment showing a strong yield response with increasing rotation complexity, particularly for corn, a comparison of NPP across a full crop rotation shows a different pattern. I used grain harvest data collected on this site (http://lter.kbs.msu.edu/datatables) and followed Bolinder et al. (2007) to estimate plant C returning to soil from aboveground non-harvested biomass, root tissue, and root deposition for each crop type to determine mean annual NPP across a full crop rotation cycle (e.g. total C returned to soil from the mean of a CSW rotation vs. three years of cC). Mean NPP calculated for 2008 through 2010 specifically for plots examined in this study differed little from a calculation of six years of mean annual NPP across all phases of the rotations occurring on the experimental site (supplemental Fig. 2.4). For 2010, the rotations with the lowest mean annual NPP were cS and cC (1799 and 1836 kg C ha^{-1} yr^{-1}, respectively) while CS was 2751 kg C ha^{-1} yr^{-1}, which was similar to CSW1cov and CSW2cov (2677 and 2811 kg C ha^{-1} yr^{-1}, respectively).

From the same data archive I estimated C inputs from weed and cover crop biomass, although these time points were only in the fall (e.g. collected as aboveground NPP) and with limited specificity of inputs by different plant species. The mean ANPP estimate for non-grain crop (weed+cover crop) biomass ranged from a low of 382 kg C ha^{-1} yr^{-1} in CS to a high of 677 kg C ha^{-1} yr^{-1} in CSW2cov (supplemental Fig. 2.4) with a mean across all rotations of 552 kg C ha^{-1} yr^{-1}, and of the rotations with cover crops, only 13% of the biomass was identified as clover

cover crop species. Among no-cover rotations, including C inputs from both grain and auxiliary plants, the CS rotation had between 22-26% greater residue returns than other treatments (cS, cC, CSW). Compared to rotations with cover, CS had 4% less C inputs than CSW1cov and 10% less than CSW2cov. Given this range of residue inputs both among no-cover rotations and between cover and no-cover treatments, the contrast in belowground C processing, transformation, and respiration detailed in this study is striking. These results suggest that the input of cover crops over the winter (e.g., as living, active roots) and into spring prior to plowing, is a primary factor driving microbial activity and SOC dynamics.

Rotations with cover crops sustained higher baseline labile C pools in soil, in spite of similar seasonal fluctuations for all treatments over the growing season (Fig. 2.1). This pool of labile C is indicative of active microbial metabolism (Fierer and Schimel, 2003), reflects changes in management and environmental conditions (Ladoni et al., 2015), and is strongly correlated with soil microbial biomass C (Franzluebbers et al., 2000). Root inputs from cover crops have been shown to contribute larger C inputs to soil than aboveground biomass (Kong and Six, 2010; Rasse et al., 2005), and likely shaped mineralization patterns in this study. Microbial biomass has a similar ratio of nutrient content (C:N:P) across a large range of aboveground ecosystems and inputs (Kallenbach and Grandy, 2011) and when normalized to PMC, hydrolytic enzyme activities did not differ across treatments (supplemental Fig. 2.1), indicating that these enzyme activities were tied to the relative size of labile C pools across all treatments. Hydrolytic enzymes for C, N and P acquisition (BG, NAG+TAP, PHOS, respectively) were all significantly higher in the cover cropped treatments (Table 2.3), and sustained higher enzyme potentials throughout the growing season for both study years (Fig. 2.2).

The ratios of hydrolytic to oxidative enzyme potentials (PEROX and PHENOX) reflect that a metabolic shift in microbial C use occurred between treatments with cover crops compared to those without cover (Table 2.4) toward a disproportionate breakdown of more stable or recalcitrant C substrates (Sinsabaugh, 2010). Different C substrate acquisition patterns by microbes in the no-cover treatments appeared to be less related to plant litter chemistry (e.g. comparing cS and cC) than to a general response to oxidize recalcitrant C to acquire nutrients or energy. PMC values and patterns in microbial C acquisition over both growing seasons suggest a means by which total SOC diverged between cover and no-cover treatments. Higher mineralization potential in the cover crop treatments indicated that a greater proportion of respired C derived from labile pools, and that a greater flux of microbially processed C into a stable pool bond to mineral soil (Cotrufo et al., 2013; Kallenbach et al., 2015, 2016). By contrast, over two years under the no-cover treatments the microbial community allocated resources that favored recalcitrant SOC oxidation resulting in a greater amount of respired C derived from degrading SOC pools that might otherwise be stable under cover crops.

Apart from distinct metabolic patterns in soil C use between cover and no-cover treatments, root inputs from cover crops may facilitate other SOC pathways. On the same site, Tiemann et al. (2015) found significantly greater aggregate formation under cover cropped treatments, in spite of regular tillage across all treatments during the growing season. Greater physical protection of C in soil aggregates preserves plant C from oxidative degradation (Grandy and Robertson, 2007) more so than chemical recalcitrance (Dungait et al., 2012; Rasse et al., 2005), which would bolster a more passive pathway for SOC accrual. In addition, root C that passes into microbial biomass can ascend rapidly through the soil food web (Bradford et al.,

2012) resulting in a larger trophic cascade (Borer et al., 2005) under cover crops and an augmented flux of root C back into labile PMC pools.

In spite of a broad range in the amount of inputs from NPP across rotations, only treatments under cover crops gained more SOC while respiring more CO_2 (Table 2.5). Mean *in situ* CO_2 fluxes in no-cover treatments were 21% and 20% less compared to treatments with cover crops during each respective cropping season. A temporal component may influence this outcome as the largest divergence in respiration rates occurred in the earlier parts of each season (Fig. 2.5, supplemental Table 2.2). Also, the source of respired C may be distinct for cover and no-cover treatments. While recalcitrant soil C is disproportionately metabolized in no-cover treatments, greater soil aggregation under cover crops may spatially structure soil habitat in a way that determines which soil C pools are more readily respired. Soils with high SOC and larger labile C pools may have slower decay constants – thus lower respiration per unit SOC, due to physical protection (Colman and Schimel, 2013). Finally even though the majority of time points showed no significant difference between fluxes, microbial C use efficiency may also differ (Sinsabaugh et al., 2013) such that the conversion of plant and soil C into microbial matter and energy differs between cover and no-cover treatments (Kallenbach et al., 2015). Differences in C use efficiency between treatments could be due to substrate biochemistry (e.g. legume inputs) or the physiology of microbial growth (Lee and Schmidt, 2014), or both litter quality and microbial physiology.

Inclusion of cover crops was the greatest factor in altering belowground ecosystem processes that underpin differences in agronomic performance across the treatments in this study. Constraining the measured soil parameters into a matrix-based ordination analysis reveals that the differences in soil ecosystem processes between continuous monocultures and the most

complex rotations are best explained by oxidative enzyme potentials (PHENOX and PEROX) at one extreme, and parameters relating to potentially mineralizable N (PMN) and NAG (Fig. 2.5) at the other. In addition to altered microbial C metabolism, cover cropped treatments had nearly double the magnitude of PMN compared to no-cover rotations (supplemental Table 2.1). Along with similar patterns in PMC and hydrolytic enzyme activities, the soils in the cover cropped treatments sustained nutrient mineralization potentials throughout the growing season that explained greater potential for root uptake and crop growth.

Synthesis

Our conceptual model (Fig. 2.6) attempts to bridge values of SOC – which are a reflection of long term management history – with measures of C pools and fluxes, and microbial metabolic activity, that turnover much more quickly. Monoculture plots had significantly less SOC than plots with cover crops (7.95 and 9.34 g C kg soil^{-1}, respectively). Complex rotations received more C inputs and respired more CO_2, but also led to SOC accumulation over time. Two other measurements showed consistent differences between cover and no-cover treatments. First, mineralizable C was significantly higher under cover crops, indicating a larger stock of C that is poised for microbial processing (i.e., as this C is processed, more is turned into protected C *and* more is respired). Second, enzyme activity also reflected different patterns in microbial C metabolism between the cover and no-cover treatments. In general, we found that hydrolytic enzymes for C, N and P acquisition were similar across treatments when normalized to PMC – thus reflecting a similar proportional allocation to sustain relative sizes of the microbial biomass pool. In contrast, the oxidative enzyme activity was much higher in monoculture plots, reflecting greater accessing of more stable C, associated with mineral-bound C. This reflects a different pattern in metabolic C acquisition along the gradient, with the monoculture plots more

reliant on recalcitrant forms of SOC, and the cover cropped plots less so, which should be reflected in both the resulting stores of SOC and fluxes of respired CO_2. The presence of cover crops has been shown to increase SOC over time (Kong and Six, 2010; Syswerda et al., 2011) even compared to rotations with greater NPP, and this work indicates possible mechanisms for soil C processing which could explain this finding.

Implications

The crop rotations in this study do not represent the dominant row crop management systems found in the U.S. Midwest. For example, continuous monoculture corn (cC) would not be grown in the absence of substantial fertilizer inputs (which would greatly increase NPP), and the most diverse rotations in this experiment (CSW2cov) are much more rare on farms due to present socioeconomic challenges for farmers including labor and energy costs (Davis et al., 2012). However, cropping systems similar to these do fall within the spectrum of agroecosystem management that both sustain grain yields and lead to improvements in ecosystem services (Robertson et al., 2014). More importantly, the more complex rotations represent an alternative to the common winter bare fallow practice used in most Midwestern cropping systems, which can lead to soil C degradation pathways (Nunan et al., 2015).

Adding cover crops into row crop agriculture has multiple effects on soil ecosystem function, including increased N supplied to crops (Tonitto et al., 2006) and reduced downstream impacts from leaching (Plaza-Bonilla et al., 2015). This study also suggests mechanisms by which cover crops shift the flow of C by expanding a pool of readily metabolizable C and altering microbial metabolic processing of SOC. This may change the magnitude of trophic level flows of labile C (DuPont et al., 2009) and microbially processed C that is stabilized on soil

minerals (Cotrufo et al., 2015; Kallenbach et al., 2015). Our results suggest that more than crop composition *per se*, or the magnitude of C inputs from NPP, the changes in soil ecosystem function observed in cover cropped treatments may be due to: i) temporal effects of cover crop inputs over winter leading to a large labile C pool at planting in the spring, ii) spatial effects of the accessibility of cover crop residues, especially roots, to degradation iii) chemical composition of residue inputs (or lack thereof), or some combination of these three factors. The resulting soil ecosystem is poised for mineralization of nutrients from internal cycling processes, which can sustain higher crop growth and multiple ecological functions similar to more diverse natural ecosystems (Zavaleta et al., 2010).

ACKNOWLEDGEMENTS

Support for this research was provided by a U.S. Department of Agriculture Soil Processes Program, grant # 2009-65107-05961, and the National Science Foundation Long-term Ecological Research Program (DEB 1027253) at the Kellogg Biological Station.

APPENDIX

Table 2.1 Crop rotations sampled on the KBS Biodiversity Gradient Experiment

ID	Treatment	Rotation	Species / yr.	Species / rot.
1	cC	corn	1	1
2	cS	soy	1	1
3	CS	corn + soy	1	2
4	CSW	corn + soy + wheat	1-2	3
5	CSW1cov	corn + soy + wheat + clover	2-3	4
6	CSW2cov	corn + soy + wheat + clover + rye	3-4	5

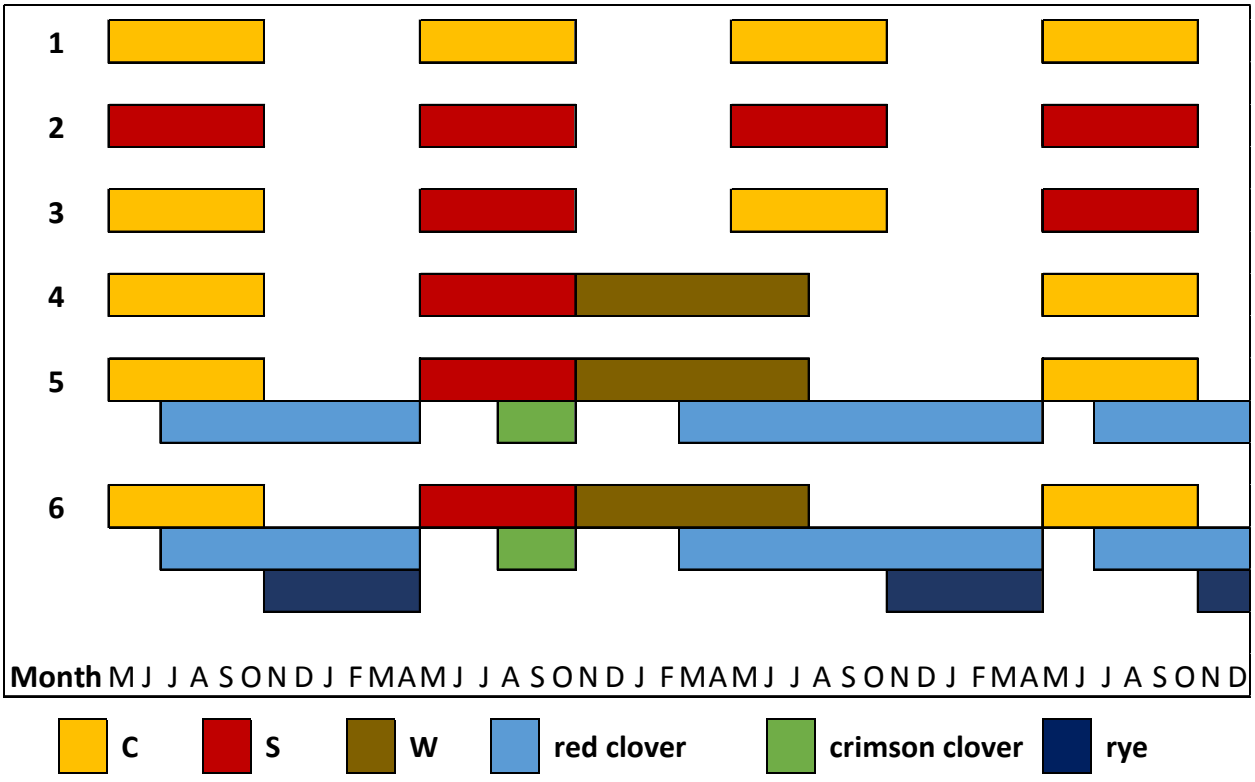


Table 2.2 Soil organic C (SOC) and N (SON) and their ratio (C:N), in the biodiversity gradient treatments (see table 2.1 for treatment acronyms). Values are means (standard error), n=4 replicate blocks. Significance of treatment effect: * $P \leq 0.05$.

Rotation	SOC g C kg soil^{-1}	SON g N kg soil^{-1}	C:N
cS	7.93(0.96)	0.80(0.09)	9.9(0.13)
cC	7.56(0.61)	0.73(0.06)	10.5(0.46)
CS	7.95(0.96)	0.76(0.12)	10.8(0.58)
CSW	7.93(0.60)	0.77(0.07)	10.5(0.54)
CSW1cov	9.30(0.35)*	0.91(0.05)*	10.3(0.45)
CSW2cov	9.38(0.38)*	0.93(0.06)*	10.2(0.38)

Table 2.3 Beta-glucosidase (BG), N-acetyl-glucosaminidase (NAG), tyrosine amino peptidase (TAP), acid phosphatase (PHOS), phenol oxidase (PHENOX),peroxidase (PEROX) in the biodiversity gradient treatments (see table 2.1 for treatment acronyms) for the two study years. Values are means (standard error), n=4 replicate blocks. Significance of treatment effect: * $P \leq 0.05$, ** $P = 0.01$ to 0.001. Each year was analyzed separately.

Year	Rotation	BG nmol g^{-1} h^{-1}	NAG nmol g^{-1} h^{-1}	TAP nmol g^{-1} h^{-1}	PHOS nmol g^{-1} h^{-1}	PHENOX µmol g^{-1} h^{-1}	PEROX µmol g^{-1} h^{-1}
2010	cS	59.9 (5.7)	17.0(1.7)	10.1(2.3)	160.4(44.2)	1.1(0.1)	2.7(0.4)
	cC	58.8 (5.8)	18.9(1.6)	8.4(1.5)	123.2(20.8)	1.0(0.2)	2.0(0.5)
	CS	68.9 (8.5)	20.9(2.2)	9.6(1.8)	128.1(18.1)	0.9(0.2)	2.0(0.4)
	CSW	63.0 (6.5)	23.3(2.7)	7.7(1.3)	118.0(22.2)	0.9(0.2)	2.2(0.3)
	CSW1cov	91.7(11.2)*	33.1(3.3)**	11.6(3.1)*	182.4(32.8)**	0.8(0.2)	1.7(0.3)
	CSW2cov	98.5 (9.8)**	38.5(4.1)**	12.0(2.6)*	171.3(26.9)**	1.2(0.2)	1.6(0.3)
2011	cS	67.9 (6.9)	21.0(2.4)	17.6(3.1)	96.5 (8.1)	3.2(0.6)	6.0(0.7)
	cC	75.4 (9.8)	25.1(2.5)	13.4(3.3)	104.8(12.3)	3.4(0.7)	6.4(0.7)
	CS	66.9 (7.9)	20.5(2.6)	11.8(1.8)	96.1(11.1)	2.3(0.3)	5.3(0.7)
	CSW	66.6 (7.1)	23.3(2.9)	17.2(4.3)	110.5(14.9)	2.4(0.3)	5.2(0.7)
	CSW1cov	92.3 (9.6)**	34.7(4.2)*	18.9(3.5)	151.4(14.6)**	2.8(0.4)	4.6(0.5)
	CSW2cov	95.0 (9.0)**	40.7(6.6)**	20.5(3.1)	138.4(14.6)**	2.5(0.4)	5.4(0.5)

Table 2.4. Potentially mineralizable C (PMC), ratio of phenol oxidase and peroxidase (OX) to PMC (OX:PMC), to beta-glucosidase BG:OX, to N-acetyl-glucosaminidase and tyrosine amino peptidase (NAG+TAP:OX), to acid phosphotase (PHOS:OX), in the biodiversity gradient treatments (see table 2.1 for treatment acronyms) for the two study years. Values are means (standard error), n=4 replicate blocks. Significance of treatment effect: * $P \leq 0.05$, ** $P = 0.01$ to 0.001. Each year was analyzed separately.

Year	Rotation	PMC $\mu g\ CO_2\text{-}C\ g^{-1}$	OX:PMC	BG:OX	NAG+TAP:OX	PHOS:OX
2010	cS	31.3(3.6)	0.16(0.03)	22.9 (4.5)	9.1 (1.7)**	62.9(18.9)
	cC	29.3(3.8)	0.10(0.01)	36.7(12.1)	16.4(5.2)	76.4(25.2)
	CS	36.0(5.5)	0.13(0.03)	32.3 (6.1)	14.1 (2.6)	62.7(13.0)
	CSW	37.4(5.7)	0.09(0.01)	30.0 (6.1)	14.0 (2.5)	60.6(17.0)
	CSW1cov	55.4(6.8)*	0.05(0.01)**	57.4(14.5)*	26.8 (6.6)*	113.2(27.2)*
	CSW2cov	52.9(6.8)*	0.06(0.01)**	56.2(14.9)*	27.6 (7.1)*	114.8(41.0)*
2011	cS	32.6(3.6)	0.31(0.04)	9.5 (1.7)	5.1 (0.8)	14.4 (3.1)
	cC	29.4(2.7)	0.33(0.03)	10.6 (2.2)	5.1 (0.8)	13.5 (2.2)
	CS	34.5(3.3)	0.24(0.03)*	12.3 (2.7)	5.4 (0.9)	17.2 (3.6)
	CSW	31.9(2.8)	0.28(0.04)	10.2 (1.7)	5.8 (0.9)	16.5 (2.9)
	CSW1cov	47.6(4.4)*	0.16(0.01)**	13.5 (1.5)	7.7 (0.7)*	22.7 (2.8)*
	CSW2cov	45.8(3.8)*	0.19(0.02)**	14.3 (2.3)	8.5 (1.0)**	21.1 (3.8)*

Table 2.5 *In situ* carbon dioxide fluxes in the biodiversity gradient treatments (see table 2.1 for treatment acronyms) for the two study years. Median, maximum (Max) and mean (standard error) for n=4 replicate blocks. Significance of treatment effect: * $P \leq 0.05$, ** $P = 0.001$ to 0.01, *** $P \leq 0.001$. Each year was analyzed separately.

Year	Rotation	Median	Max	Mean (SE)
		kg CO_2-C - ha^{-1}-day^{-1}		
2010	cS	21.8	43.2	22.2 (2.4) **
	cC	26.3	87.1	33.8 (4.7)
	CS	30.9	86.2	35.8 (3.9)
	CSW	29.5	73.8	34.9 (3.8)
	CSW1cov	31.1	100.3	39.1 (5.4)
	CSW2cov	31.1	90.1	41.2 (4.8) *
2011	cS	13.4	22.7	14.1 (1.2)
	cC	14.6	21.3	14.0 (1.1)
	CS	13.7	23.3	14.4 (1.1)
	CSW	12.6	21.1	13.5 (1.0)
	CSW1cov	16.2	23.5	16.2 (1.0) **
	CSW2cov	20.0	30.7	18.8 (1.3) ***

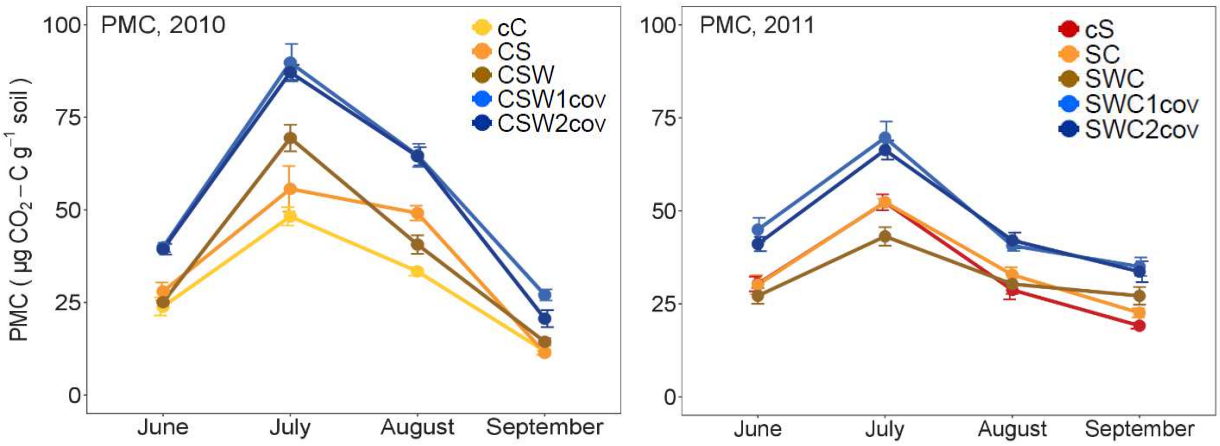


Figure 2.1. Potentially mineralizable C (PMC) in the biodiversity gradient treatments (see table 2.1 for treatment acronyms) for a corn year (2010) and a soybean year (2011). Values are means with standard error for each month, n=4 replicate blocks.

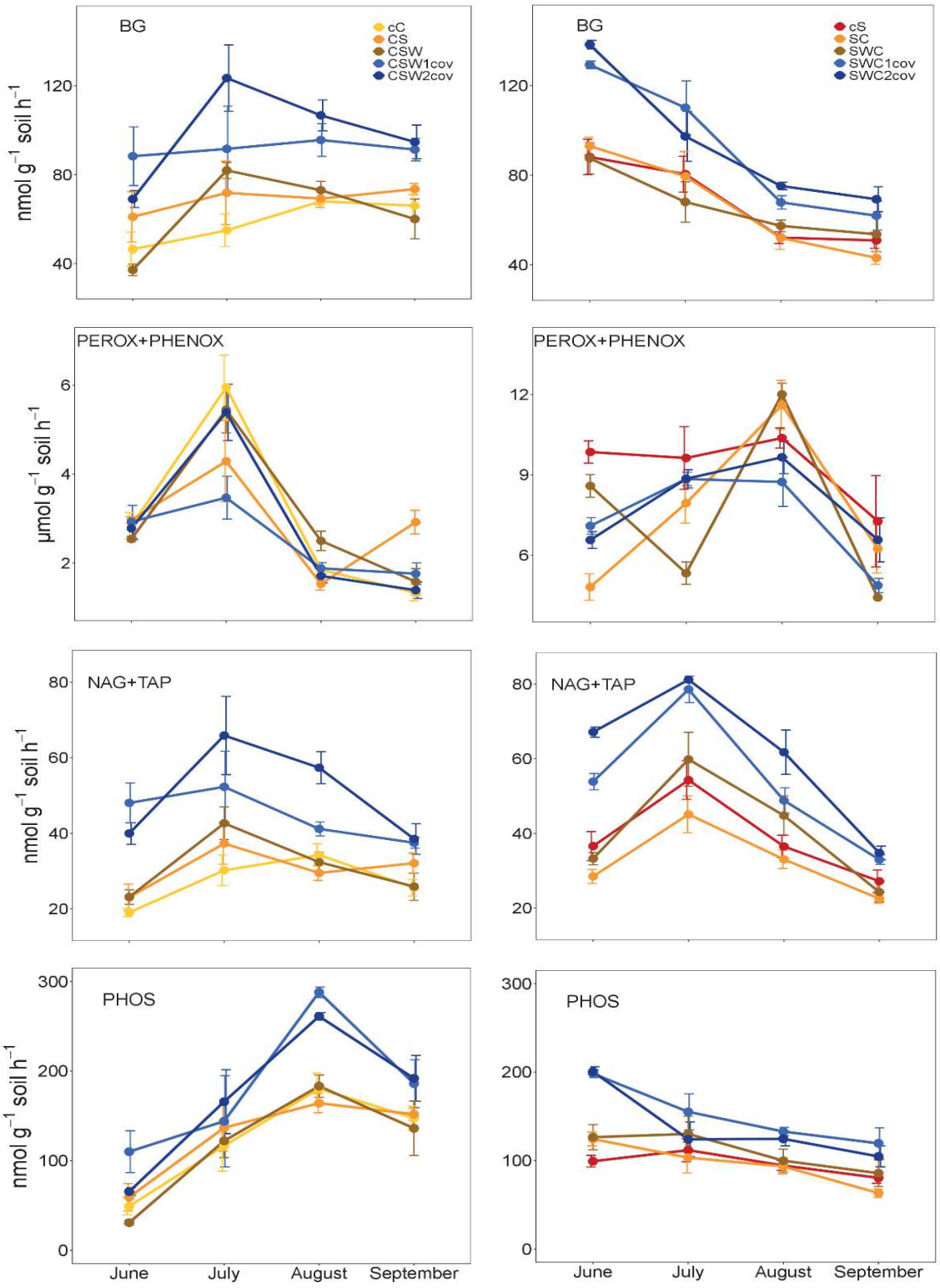


Figure 2.2. Enzyme activities for beta-glucosidase (BG), peroxidase (PEROX) + phenol oxidase (PHENOX), N-acetyl-glucosaminidase (NAG) + tyrosine amino peptidase (TAP), acid phosphatase (PHOS). All points are means across blocks (n=4) with standard errors for a corn year (left) and following soybean year (right). See table 2.1 for treatment acronyms.

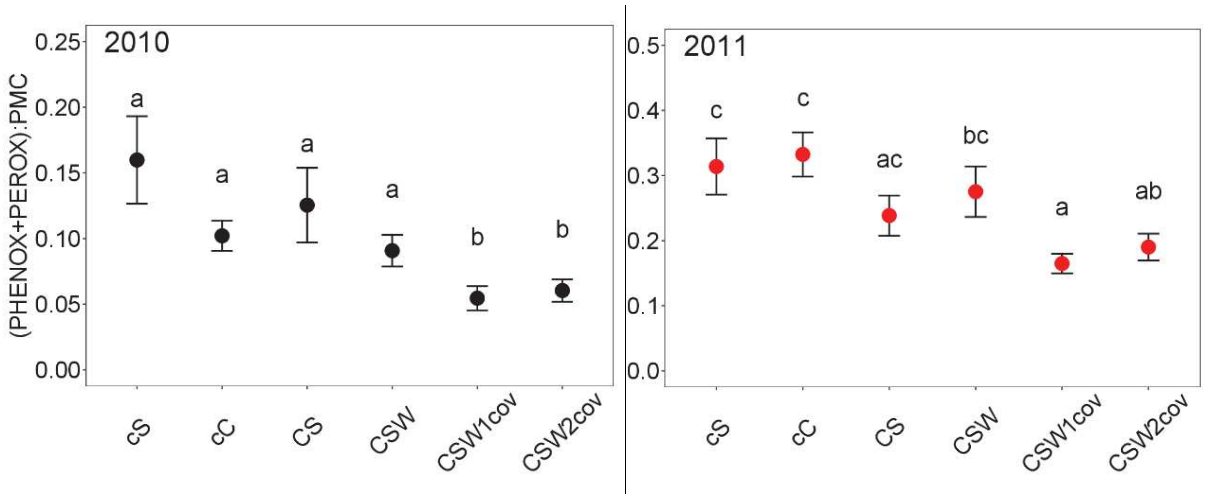


Figure 2.3. Ratio of soil phenol oxidase (PHENOX) + peroxidase (PEROX) enzyme activities to potentially mineralizable carbon (PMC). All points are means across blocks (n=4) with standard errors for a corn year (left) and following soybean year (right). Letters indicate statistical difference ($P \leq 0.05$) based on *post hoc* Tukey's test. See table 2.1 for treatment acronyms.

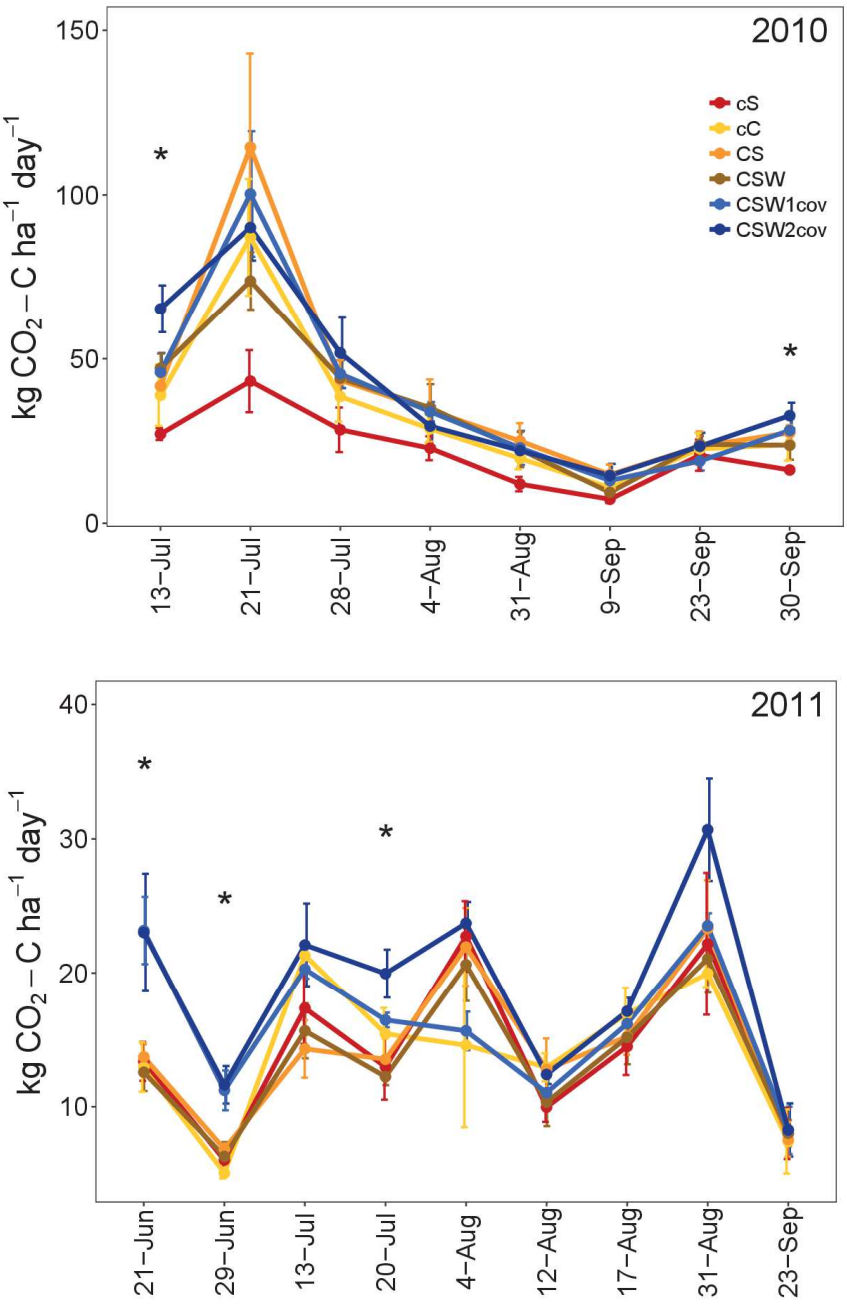


Figure 2.4. *In situ* carbon dioxide flux measurements for KBS biodiversity gradient (see table 2.1 for treatment acronyms) for two years. Time points are means with standard error, n=4. Asterisks (*) show significant differences ($P \leq 0.05$) by treatments at a specific time point. In 2010, cS was left out of treatment comparisons, as was cC in 2011.

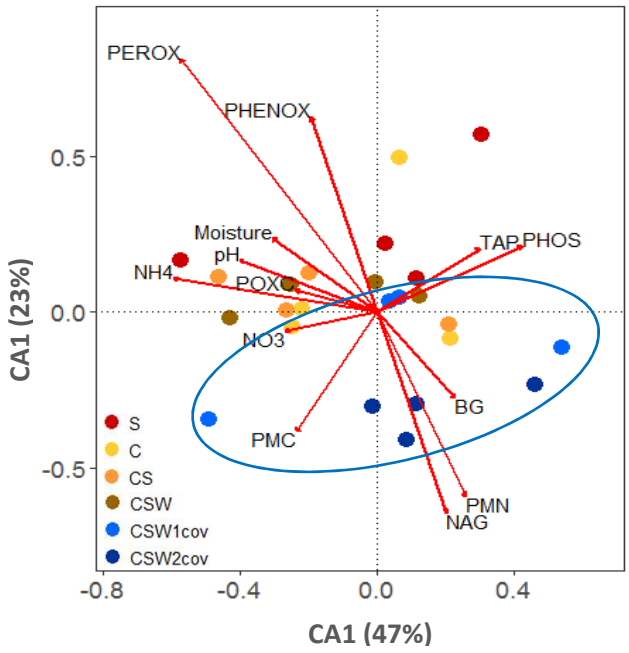


Figure 2.5. Canonical correspondence analysis plot of KBS biodiversity gradient for combined two years of study with differences in rotations constrained to measured soil parameters and the two axes representing the greatest explained variation (on a percentage basis). Blue ellipse encircles treatments with cover crops. Figures are from cumulative measures over the season for all parameters except pH (which represents a mean value). Other parameters are Moisture (soil moisture), inorganic N (NH_4^+ and NO_3^-), potentially mineralizable N (PMN) and C (PMC), permanganate oxidizable C (POXC), enzyme activities for beta-glucosidase (BG), peroxidase (PEROX) + phenol oxidase (PHENOX), N-acetyl-glucosaminidase (NAG) + tyrosine amino peptidase (TAP), acid phosphatase (PHOS). All ordinations were created with a correlational matrix to give equal weights to all soil parameters. See table 2.1 for treatment acronyms.

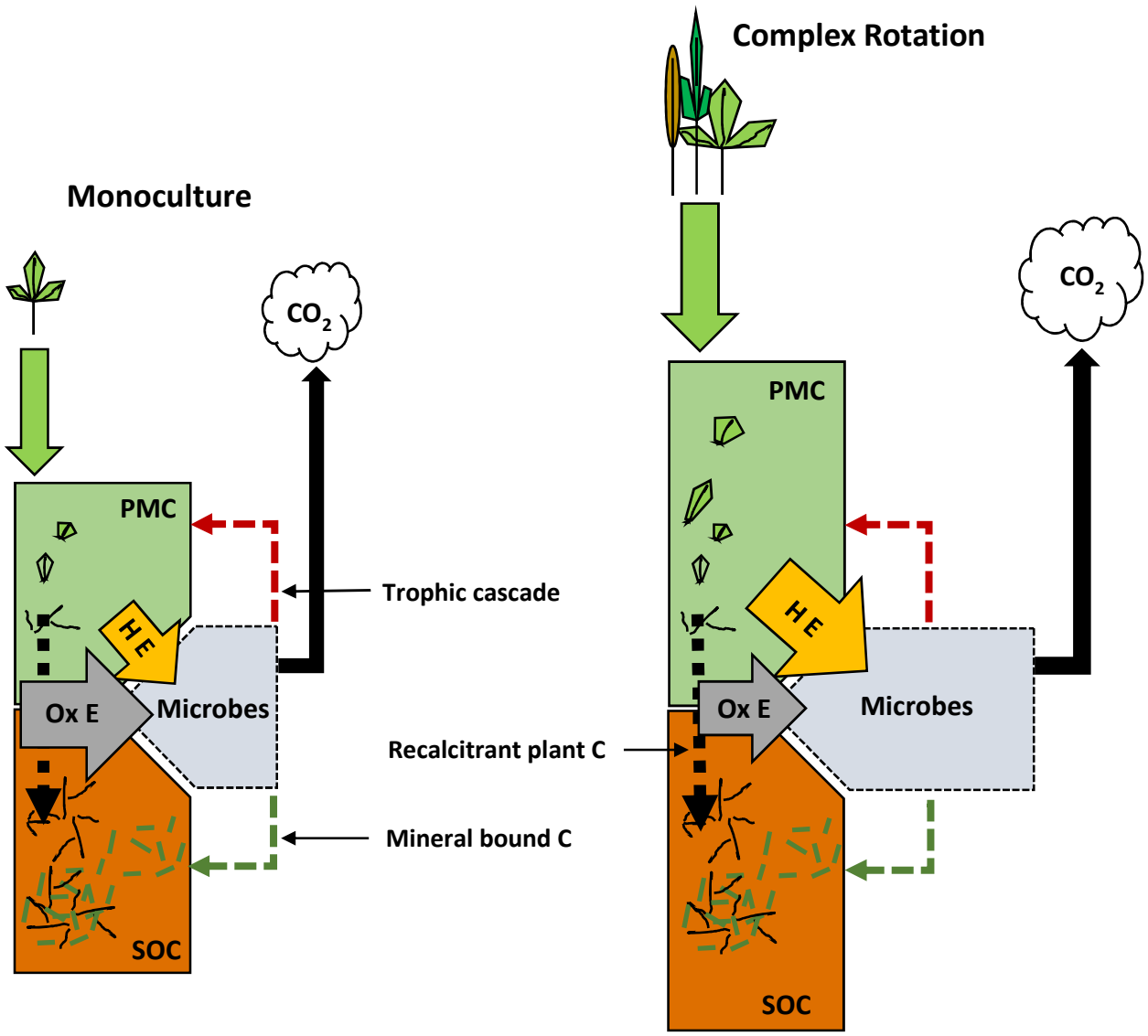


Figure 2.6. Model for C transformation in contrasting continuous 'Monoculture' (cC) and 'Complex Rotations' (CSW2cov) on the KBS biodiversity gradient experiment. Boxes and solid arrows for specific parameters are in relative proportion to measured values from this study. Dashed arrows represent posited, but unknown C flows. Green arrows at the top are plant inputs entering a labile pool of potentially mineralizable C (PMC). Yellow arrows represent the magnitude of hydrolytic enzyme activity (H E) and gray arrows are oxidative enzyme activities (Ox E), which draw soil C substrates into microbes (gray box). Passing out of microbial cells is respired CO_2 (black arrow) as well as some unknown quantity of C that passes back into a labile C fraction via a trophic cascade (red dashed arrow) and microbial-processed C which complexes with soil as 'Mineral bound C' (dark green dashed arrow, also unknown). Stable C which forms the bulk of SOC (light brown box) derives both from microbially-processed C and from 'Recalcitrant plant C' (dashed black arrow) which is not readily accessed by microbial activity and passes into longer term SOC pools.

Supplemental Table 2.1 Inorganic N (NH_4^+-N and NO_3^--N) and potentially mineralizable N (PMN) in the biodiversity gradient treatments (see table 2.1 for treatment acronyms) for the two study years. Values are means (standard error), n=4 replicate blocks. Significance of treatment effect: * $P \leq 0.05$, ** $P = 0.001$ to 0.01. Each year was analyzed separately.

Year	Rotation	NH_4^+ μg NH_4^+-N g^{-1}	NO_3^- μg NO_3^--N g^{-1}	PMN μg NH_4^+-N g^{-1}
2010	cS	2.5(0.6)	2.9(0.5)	5.2(0.6)
	cC	1.7(0.4)	1.3(0.3)	4.0(0.5)
	CS	1.2(0.2)	0.9(0.1)	5.3(0.6)
	CSW	2.2(0.6)	1.2(0.3)	5.2(0.6)
	CSW1cov	3.1(0.7)*	3.6(0.8)**	8.7(0.7)*
	CSW2cov	1.9(0.2)	2.4(0.4)*	9.0(0.8)*
2011	cS	1.7(0.3)	2.4(0.3)	4.1(0.5)
	cC	1.7(0.2)	1.6(0.2)	3.6(0.4)
	CS	1.9(0.3)	2.5(0.2)	4.0(0.5)
	CSW	1.7(0.2)	2.1(0.2)	4.9(0.6)
	CSW1cov	2.3(0.4)	3.6(0.6)	8.5(0.8)*
	CSW2cov	2.0(0.3)	2.5(0.3)	8.9(0.9)*

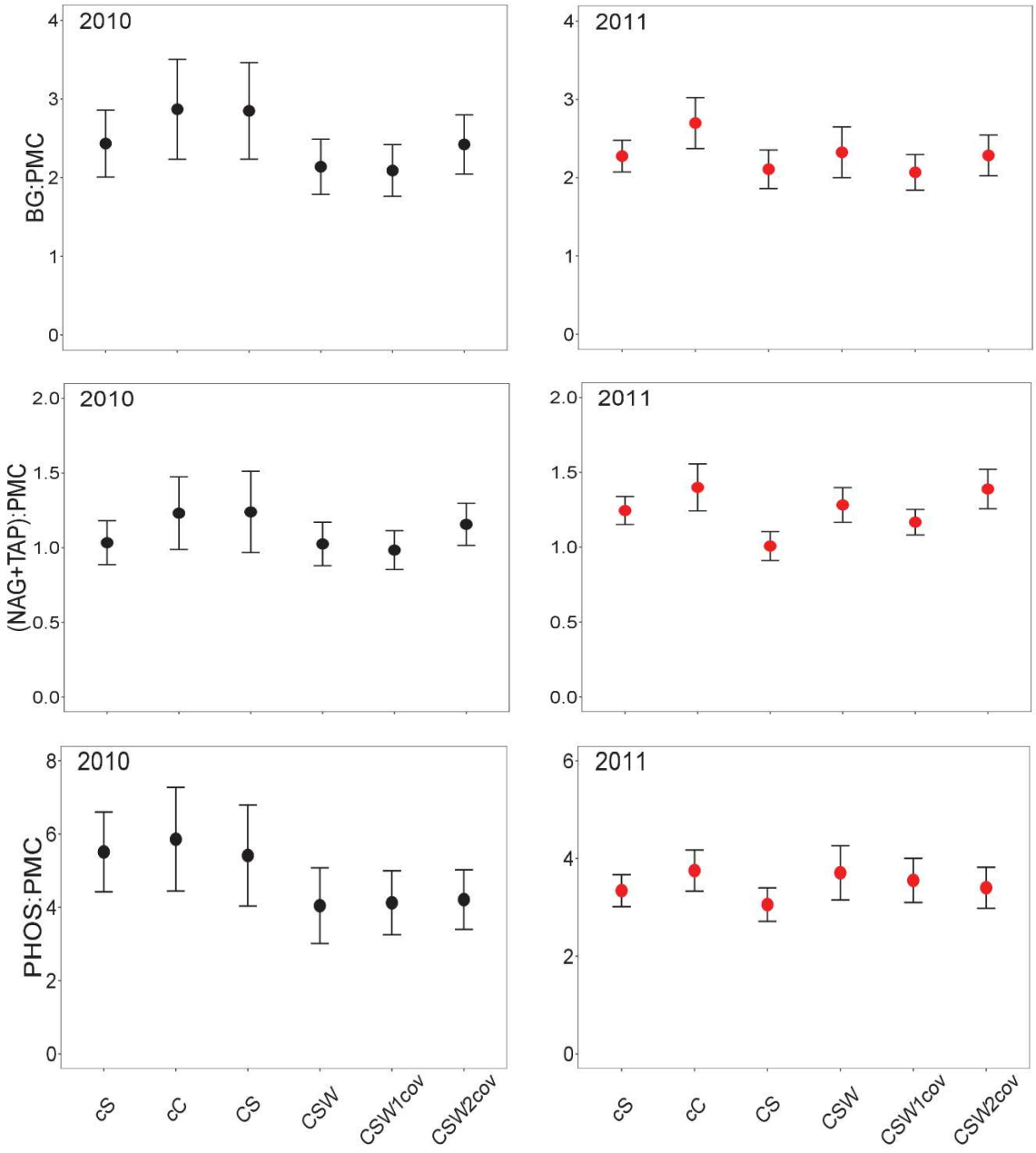
Supplemental Table 2.2. *In situ* carbon dioxide fluxes in the biodiversity gradient treatments for individual time points over two years with mean (standard error) for n=4 replicate blocks, followed by letters denoting significant differences between treatments. See table 2.1 for treatment acronyms.

2010 g CO_2-C ha^{-1} day^{-1}

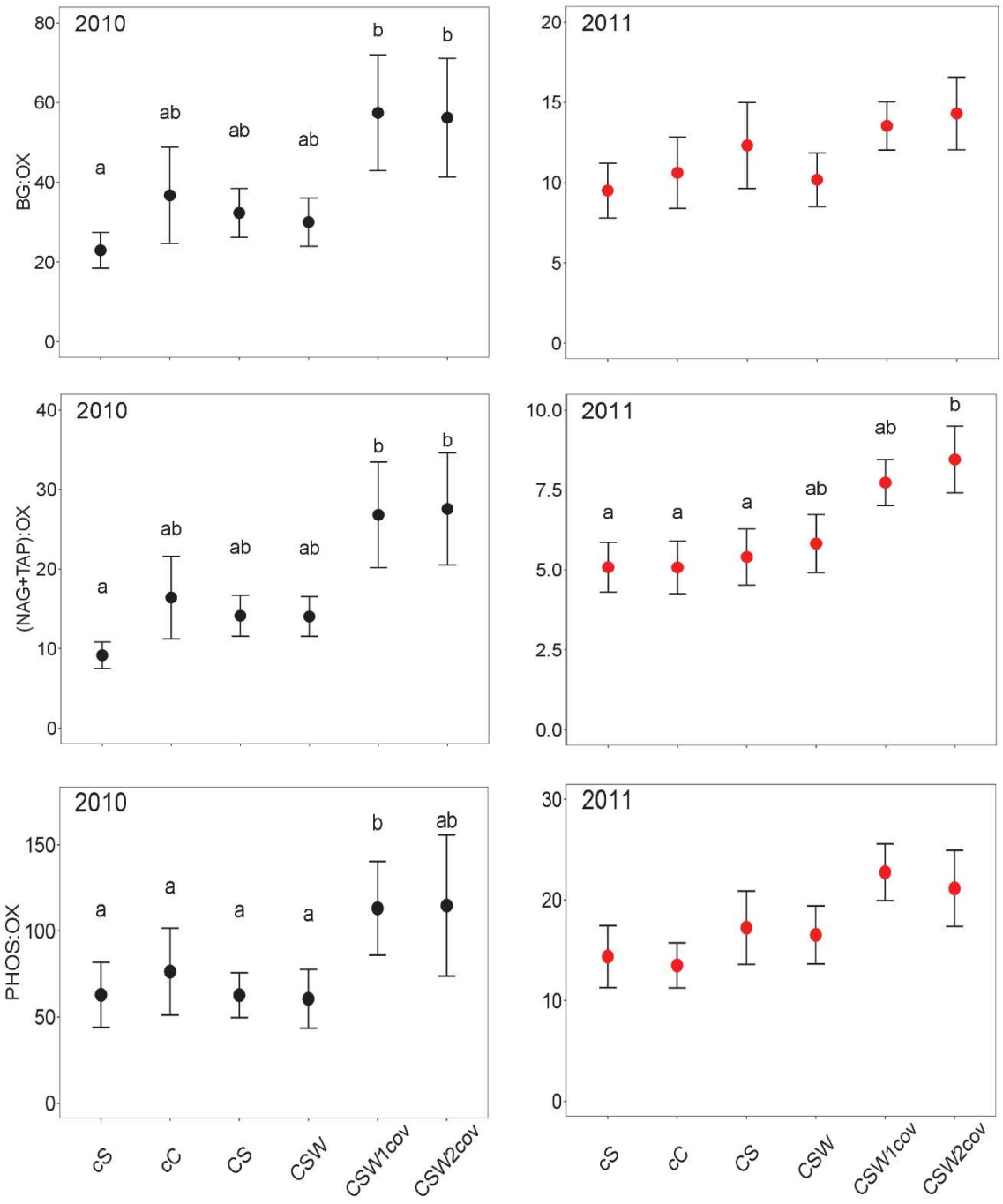
	07/13/10	07/21/10	07/28/10	08/04/10	08/31/10	09/09/10	09/23/10	09/30/10
cS	27.1 (1.8)$_a$	43.2 (9.4)	28.4 (6.8)	22.8 (3.6)	11.9 (2.2)	7.3 (1.1)	20.7 (4.7)	16.2 (0.6)$_a$
cC	38.9 (9.3)$_{ab}$	87.1 (17.8)	38.5 (7.6)	28.7 (4.2)	19.7 (3.3)	10.8 (1.5)	22.6 (4.8)	23.9 (4.9)$_{ab}$
CS	41.7 (3.7)$_{ab}$	114.5 (28.4)	43.5 (6.0)	34.3 (9.4)	25.0 (5.4)	14.8 (3.0)	23.4 (4.5)	27.5 (2.3)$_{ab}$
CSW	47.1 (4.5)$_{ab}$	73.8 (8.8)	44.1 (5.4)	35.1 (7.1)	22.4 (5.4)	9.4 (2.9)	24.0 (2.6)	23.7 (4.4)$_{ab}$
CSW1cov	45.9 (5.9)$_{ab}$	100.3 (19.1)	45.4 (4.2)	33.9 (3.0)	22.9 (5.2)	13.0 (2.3)	18.9 (2.9)	28.2 (3.6)$_{ab}$
CSW2cov	65.3 (7.2)$_b$	90.1 (10.0)	51.8 (10.8)	29.5 (6.1)	22.1 (2.1)	14.5 (3.5)	23.4 (4.1)	32.7 (3.9)$_b$

2011 g CO_2-C ha^{-1} day^{-1}

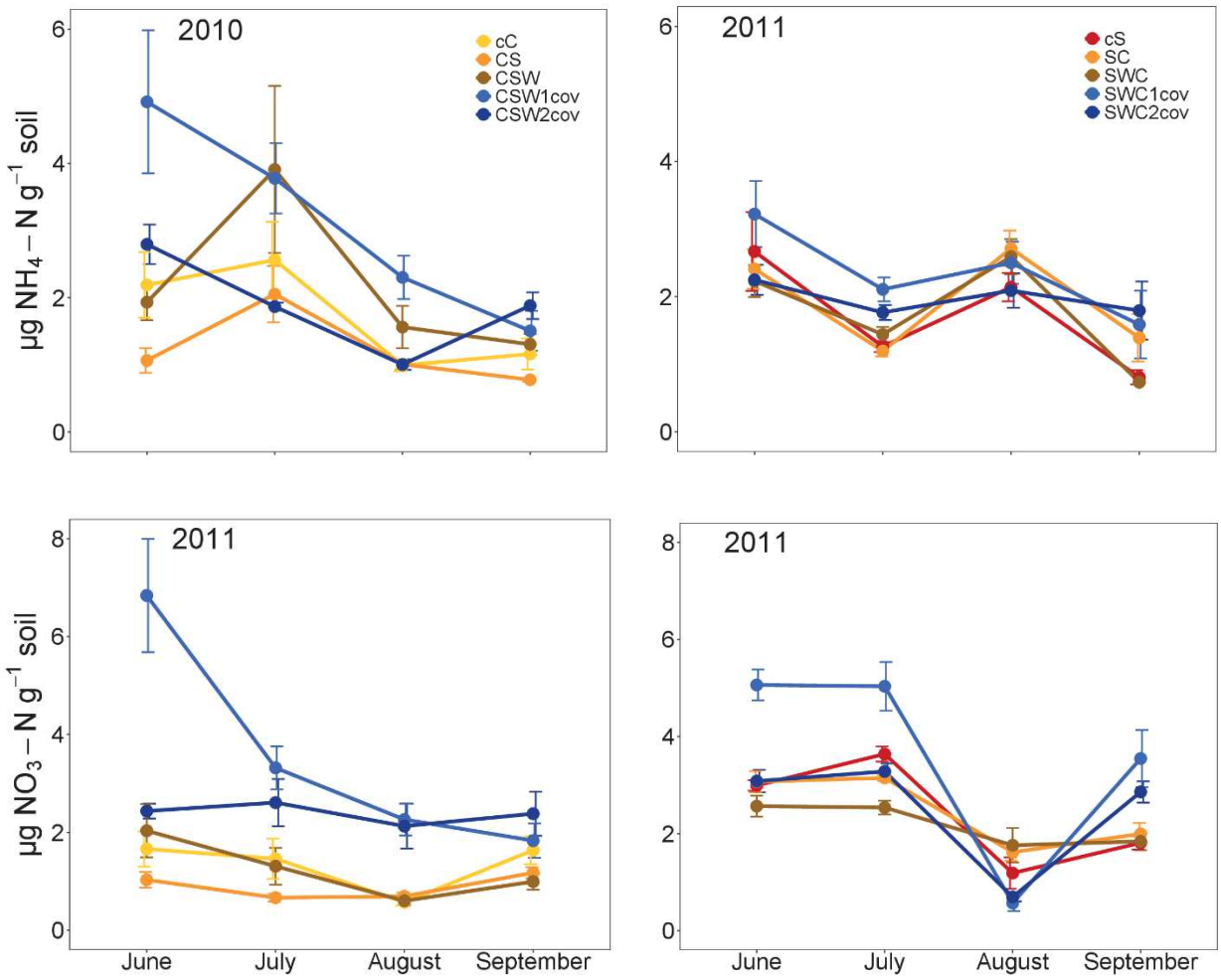
	06/21/11	06/29/11	07/13/11	07/20/11	08/04/11	08/12/11	08/17/11	08/31/11	09/23/11
cS	13.4 (1.5)$_a$	6.0 (0.9)$_a$	17.3 (2.7)	13.0 (2.4)$_a$	22.7 (2.6)	10.0 (1.1)	14.5 (2.1)	22.2 (5.3)	8.0 (1.9)
cC	12.9 (1.8)$_a$	5.1 (0.4)$_a$	21.3 (0.9)	15.4 (1.9)$_{ab}$	14.6 (6.1)	12.9 (1.0)	16.9 (1.9)	20.0 (1.0)	7.4 (2.4)
CS	13.7 (0.9)$_a$	6.8 (0.4)$_a$	14.3 (2.1)	13.5 (1.6)$_a$	21.9 (2.9)	12.8 (2.3)	15.2 (1.4)	23.3 (3.6)	7.6 (1.3)
CSW	12.6 (1.4)$_a$	6.3 (1.1)$_a$	15.7 (2.0)	12.2 (0.6)$_a$	20.6 (2.7)	10.3 (1.8)	15.2 (2.0)	21.1 (2.5)	8.0 (1.0)
CSW1cov	23.2 (2.5)$_b$	11.2 (1.5)$_b$	20.3 (0.7)	16.5 (0.5)$_{ab}$	15.6 (1.4)	11.0 (1.2)	16.2 (1.1)	23.5 (0.9)	8.2 (1.8)
CSW2cov	23.0 (4.4)$_b$	11.6 (1.4)$_b$	22.1 (3.1)	20.0 (1.8)$_b$	23.7 (1.6)	12.4 (0.7)	17.1 (1.0)	30.7 (3.8)	8.3 (2.0)



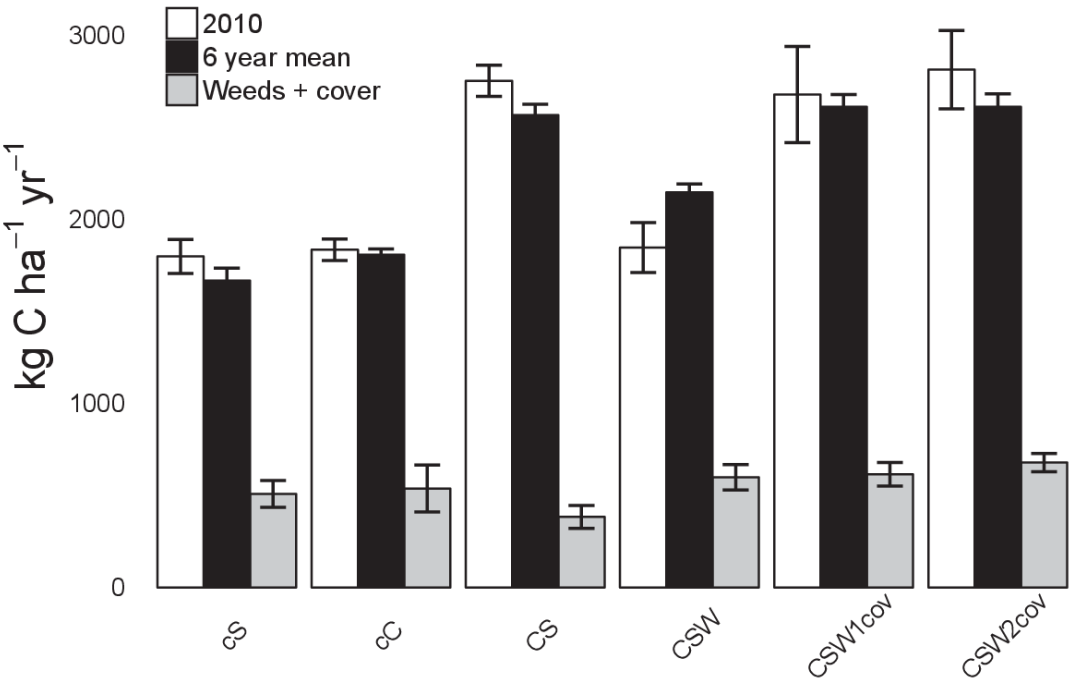
Supplemental Figure 2.1. Ratio of Potentially mineralizable C (PMC) to soil enzyme activities, beta-glucosidase, BG:PMC, to N-acetyl-glucosaminidase and tyrosine amino peptidase (NAG+TAP):PMC, to acid phosphatase, PHOS:PMC, in the biodiversity gradient treatments. See table 2.1 for treatment acronyms. All points are means across blocks (n=4) with standard errors for a corn year (left) and following soybean year (right).



Supplemental Figure 2.2 Ratio of soil phenol oxidase (PHENOX) + peroxidase (PEROX) enzyme activities to beta-glucosidase (BG:OX), to N-acetyl-glucosaminidase and tyrosine amino peptidase (NAG+TAP:OX), to acid phosphotase (PHOS:OX), in the biodiversity gradient treatments. All points are means across blocks (n=4) with standard errors for a corn year (left) and following soybean year (right). Letters indicate statistical difference (P<0.05) based on *post hoc* Tukey's test. See table 2.1 for treatment acronyms.



Supplemental Figure 2.3 Inorganic N (NH_4^+-N and NO_3^--N) in the biodiversity gradient (see table 2.1 for treatment acronyms) treatments for the two study years. All points are means across blocks (n=4) with standard errors for a corn year (left) and following soybean year (right).



Supplemental Figure 2.4 Net primary productivity (NPP) with standard error from the KBS Biodiversity Gradient Experiment. Estimated NPP calculated as mean of three years (longest period for a full rotation) for 2010 is the mean NPP of 2008, 2009 and 2010 only for the plots examined in this study. A 6 year mean is estimated NPP for all phases of each rotation from years 2008 to 2014 (each appearing every year for all treatments on the experimental site). Both these calculations are determined from the harvest index following Bolinder et al. (2007) and excluding biomass removed as harvested grain. Non-cash crop aboveground NPP (including cover crop and weeds) was also calculated from fall biomass collections. See table 2.1 for treatment acronyms.

REFERENCES

REFERENCES

Andrén, O., Bengtsson, J., Clarholm, M., 1995. Biodiversity and species redundancy among litter decomposers, in: Collins, H.P., Robertson, G.P., Klug, M.J. (Eds.), The Significance and Regulation of Soil Biodiversity, Developments in Plant and Soil Sciences. Springer Netherlands, pp. 141–151. doi:10.1007/978-94-011-0479-1_12

Bolinder, M.A., Janzen, H.H., Gregorich, E.G., Angers, D.A., VandenBygaart, A.J., 2007. An approach for estimating net primary productivity and annual carbon inputs to soil for common agricultural crops in Canada. Agriculture, Ecosystems & Environment 118, 29–42. doi:10.1016/j.agee.2006.05.013

Borer, E.T., Seabloom, E.W., Shurin, J.B., Anderson, K.E., Blanchette, C.A., Broitman, B., Cooper, S.D., Halpern, B.S., 2005. What Determines the Strength of a Trophic Cascade? Ecology 86, 528–537. doi:10.1890/03-0816

Bradford, M.A., Strickland, M.S., DeVore, J.L., Maerz, J.C., 2012. Root carbon flow from an invasive plant to belowground foodwebs. Plant Soil 359, 233–244. doi:10.1007/s11104-012-1210-y

Broussard, W., Turner, R.E., 2009. A century of changing land-use and water-quality relationships in the continental US. Frontiers in Ecology and the Environment 7, 302–307.

Clark, M.S., Horwath, W.R., Shennan, C., Scow, K.M., 1998. Changes in Soil Chemical Properties Resulting from Organic and Low-Input Farming Practices. Agronomy Journal 90, 662–671. doi:10.2134/agronj1998.00021962009000050016x

Colman, B.P., Schimel, J.P., 2013. Drivers of microbial respiration and net N mineralization at the continental scale. Soil Biology and Biochemistry 60, 65–76. doi:10.1016/j.soilbio.2013.01.003

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? Glob Change Biol 19, 988–995. doi:10.1111/gcb.12113

David, M.B., Drinkwater, L.E., McIsaac, G.F., 2010. Sources of nitrate yields in the Mississippi River Basin. Journal of Environmental Quality 39, 1657–1667.

Davis, A.S., Hill, J.D., Chase, C.A., Johanns, A.M., Liebman, M., 2012. Increasing Cropping System Diversity Balances Productivity, Profitability and Environmental Health. PLoS ONE 7, e47149. doi:10.1371/journal.pone.0047149

Doane, T.A., Horwáth, W.R., 2003. Spectrophotometric Determination of Nitrate with a Single Reagent. Analytical Letters 36, 2713–2722. doi:10.1081/AL-120024647

Drinkwater, L.E., Cambardella, C.A., Reeder, J.D., Rice, C.W., 1996. Potentially mineralizable nitrogen as an indicator of biologically active soil nitrogen, in: Methods for Assessing Soil Quality. Soil Science Society of America, Madison, WI, pp. 217–229.

Drinkwater, L.E., Wagoner, P., Sarrantonio, M., 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396, 262–265. doi:10.1038/24376

Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., Whitmore, A.P., 2012. Soil organic matter turnover is governed by accessibility not recalcitrance. Glob Change Biol 18, 1781–1796. doi:10.1111/j.1365-2486.2012.02665.x

DuPont, S.T., Ferris, H., Van Horn, M., 2009. Effects of cover crop quality and quantity on nematode-based soil food webs and nutrient cycling. Applied Soil Ecology 41, 157–167. doi:10.1016/j.apsoil.2008.10.004

Fierer, N., Schimel, J.P., 2003. A Proposed Mechanism for the Pulse in Carbon Dioxide Production Commonly Observed Following the Rapid Rewetting of a Dry Soil. Soil Sci. Soc. Am. J. 67, 798–805.

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, 613–623.

Gardner, J.B., Drinkwater, L.E., 2009. The fate of nitrogen in grain cropping systems: a meta-analysis of 15N field experiments. Ecological Applications 19, 2167–2184.

German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology and Biochemistry 43, 1387–1397. doi:10.1016/j.soilbio.2011.03.017

Grandy, A.S., Robertson, G.P., 2007. Land-Use Intensity Effects on Soil Organic Carbon Accumulation Rates and Mechanisms. Ecosystems 10, 59–74. doi:10.1007/s10021-006-9010-y

Gregorich, E.G., Drury, C.F., Baldock, J.A., 2001. Changes in soil carbon under long-term maize in monoculture and legume-based rotation. Canadian journal of soil science 81, 21–31.

Haney, R., Brinton, W., Evans, E., 2008. Soil CO2 respiration: Comparison of chemical titration, CO2 IRGA analysis and the Solvita gel system. Renewable Agriculture and Food Systems 23, 171.

Hoben, J.P., Gehl, R.J., Millar, N., Grace, P.R., Robertson, G.P., 2011. Nonlinear nitrous oxide (N2O) response to nitrogen fertilizer in on-farm corn crops of the US Midwest. Global Change Biology 17, 1140–1152. doi:10.1111/j.1365-2486.2010.02349.x

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., Wardle, D.A., 2005. Effects of Biodiversity on Ecosystem Functioning: A Consensus of Current Knowledge. Ecological Monographs 75, 3–35.

IPCC, 2013. , The Physical Science Basis. Summary for Policymakers. United Nations.

Kallenbach, C., Grandy, A.S., 2011. Controls over soil microbial biomass responses to carbon amendments in agricultural systems: A meta-analysis. Agriculture, Ecosystems & Environment 144, 241–252. doi:10.1016/j.agee.2011.08.020

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

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

Klopatek, J.M., Olson, R.J., Emerson, C.J., Joness, J.L., 1979. Land-use conflicts with natural vegetation in the United States. Environmental Conservation 6, 191–199.

Kong, A.Y.Y., Six, J., 2010. Tracing Root vs. Residue Carbon into Soils from Conventional and Alternative Cropping Systems. Soil Science Society of America Journal; Madison 74, 1201–1210.

Ladoni, M., Basir, A., Kravchenko, A., 2015. Which Soil Carbon Fraction is the Best for Assessing Management Differences? A Statistical Power Perspective. Soil Science Society of America Journal 79, 848–857. doi:10.2136/sssaj2014.10.0426

Lee, Z.M., Schmidt, T.M., 2014. Bacterial growth efficiency varies in soils under different land management practices. Soil Biology and Biochemistry 69, 282–290. doi:10.1016/j.soilbio.2013.11.012

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

Liebman, M., Helmers, M.J., Schulte, L.A., Chase, C.A., 2013. Using biodiversity to link agricultural productivity with environmental quality: Results from three field experiments in Iowa. Renewable Agriculture and Food Systems 28, 115–128. doi:10.1017/S1742170512000300

Matson, P.A., Parton, W.J., Power, A.G., Swift, M.J., 1997. Agricultural Intensification and Ecosystem Properties. Science 277, 504–509. doi:10.1126/science.277.5325.504

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2013. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecological Applications 24, 560–570. doi:10.1890/13-0616.1

Plaza-Bonilla, D., Nolot, J.-M., Raffaillac, D., Justes, E., 2015. Cover crops mitigate nitrate leaching in cropping systems including grain legumes: Field evidence and model simulations. Agriculture, Ecosystems & Environment 212, 1–12. doi:10.1016/j.agee.2015.06.014

Rabalais, N.N., Turner, R.E., Scavia, D., 2002. Beyond Science into Policy: Gulf of Mexico Hypoxia and the Mississippi RiverNutrient policy development for the Mississippi River watershed reflects the accumulated scientific evidence that the increase in nitrogen loading is the primary factor in the worsening of hypoxia in the northern Gulf of Mexico. BioScience 52, 129–142. doi:10.1641/0006-3568(2002)052[0129:BSIPGO]2.0.CO;2

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.

Robertson, G.P., Grandy, A.S., 2006. Soil System Management in Temperate Regions, in: Biological Approaches to Sustainable Soil Systems, Books in Soils, Plants, and the Environment. CRC Press, pp. 27–39.

Robertson, G.P., Gross, K.L., Hamilton, S.K., Landis, D.A., Schmidt, T.M., Snapp, S.S., Swinton, S.M., 2014. Farming for Ecosystem Services: An Ecological Approach to Production Agriculture. BioScience 64, 404–415. doi:10.1093/biosci/biu037

Robertson, G.P., Hamilton, S.K., 2015. Long-term ecological research in agricultural landscapes at the Kellogg Biological Station LTER site: conceptual and experimental framework, in: The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability. Oxford University Press, New York, New York, USA, pp. 1–32.

Robertson, P.G., Vitousek, P.M., 2009. Nitrogen in Agriculture: Balancing the Cost of an Essential Resource. Annual Review of Environment and Resources 34, 97–125. doi:10.1146/annurev.environ.032108.105046

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 & Environment 162, 68–76. doi:10.1016/j.agee.2012.08.011

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

Shcherbak, I., Millar, N., Robertson, G.P., 2014. Global metaanalysis of the nonlinear response of soil nitrous oxide (N2O) emissions to fertilizer nitrogen. PNAS 111, 9199–9204. doi:10.1073/pnas.1322434111

Shennan, C., 2008. Biotic interactions, ecological knowledge and agriculture. Philosophical Transactions of the Royal Society of London B: Biological Sciences 363, 717–739. doi:10.1098/rstb.2007.2180

Sinsabaugh, R.., Reynolds, H., Long, T.., 2000. Rapid assay for amidohydrolase (urease) activity in environmental samples. Soil Biology and Biochemistry 32, 2095–2097. doi:10.1016/S0038-0717(00)00102-4

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

Sinsabaugh, R.L., Carreiro, M.M., Alvarez, S., 2002. Enzyme and microbial dynamics of litter decomposition. Enzymes in the Environment, Activity, Ecology, and Applications. Marcel Dekker, New York, Basel 249–265.

Sinsabaugh, R.L., Hill, B.H., Follstad Shah, J.J., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462, 795–798. doi:10.1038/nature08632

Smith, C.M., David, M.B., Mitchell, C.A., Masters, M.D., Anderson-Teixeira, K.J., Bernacchi, C.J., DeLucia, E.H., 2013. Reduced nitrogen losses after conversion of row crop agriculture to perennial biofuel crops. Journal of environmental quality 42, 219–228.

Smith, R., Gross, K., Robertson, G., 2008. Effects of Crop Diversity on Agroecosystem Function: Crop Yield Response. Ecosystems 11, 355-366–366.

Syswerda, S.P., Corbin, A.T., Mokma, D.L., Kravchenko, A.N., Robertson, G.P., 2011. Agricultural Management and Soil Carbon Storage in Surface vs. Deep Layers. Soil Science Society of America Journal 75, 92–101. doi:10.2136/sssaj2009.0414

Tiemann, L.K., Grandy, A.S., Atkinson, E.E., Marin-Spiotta, E., McDaniel, M.D., 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. Ecol Lett 18, 761–771. doi:10.1111/ele.12453

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T., Lehman, C., 2001. Diversity and Productivity in a Long-Term Grassland Experiment. Science 294, 843–845. doi:10.1126/science.1060391

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

Wardle, D.A., 2002. Communities and ecosystems: linking the aboveground and belowground components. Princeton Univ Pr.

Wood, S.L.R., Rhemtulla, J.M., Coomes, O.T., 2016. Cropping history trumps fallow duration in long-term soil and vegetation dynamics of shifting cultivation systems. Ecol Appl n/a-n/a. doi:10.1002/eap.1462

Zavaleta, E.S., Pasari, J.R., Hulvey, K.B., Tilman, G.D., 2010. Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. PNAS 107, 1443–1446. doi:10.1073/pnas.0906829107

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1, 3–14. doi:10.1111/j.2041-210X.2009.00001.x

CHAPTER 3: COVER CROPS CHANGE SOIL BACTERIAL COMPOSITION BUT NOT DIVERSITY

ABSTRACT

In natural systems a strong relationship exists between aboveground biodiversity and numerous ecosystem functions. Here I examined how increased crop diversity in row crop rotations affects the diversity and composition of soil bacteria. I predicted that inclusion of more crops in rotation, including overwintering cover crops, would increase bacterial diversity in tandem with increases in soil ecosystem function. Along a gradient of increasing crops in rotation and inclusion of cover crops, we sampled 16S rDNA from soil bacterial communities. Sequencing and screening revealed a range of 3275 and 3459 distinct operational taxonomic units (OTUs) among distinct rotations. I found no effect of crop rotation on bacterial richness or diversity, but a strong divergence in bacterial community composition in rotations with cover crops, whereas all other rotations without cover crops were strongly similar. The change in community between rotations with and without cover fell along clear taxonomic lines. Among the top ten percent of most abundant OTUs, *Proteobacteria* dominated in the rotations with cover crops and while *Acidobacteria* predominated in rotations without cover. These communities correlated strongly to measures of carbon and nitrogen mineralization and enzyme activity, and indicated a physiological basis for the shift in community composition in response to inclusion of leguminous cover crops in rotation.

INTRODUCTION

Intensification of agriculture is a major factor leading to global environmental change (Balmford et al., 2012; Foley et al., 2011; West et al., 2014). This transformation has resulted in

large landscapes in the U.S. Midwest devoted to production of a few primary annual grain crops. Productivity in these agroecosystems is often dependent on large nonrenewable inputs and frequent soil disturbances, which contribute to soil erosion and other downstream impacts to air and water. With respect to nutrient pollution, these low plant diversity agroecosystems lack many of the soil ecosystem functions that drive stable and efficient nutrient cycling in natural ecosystems (Hooper et al., 2005). However, it is not clear if introducing greater plant diversity into agricultural systems will lead to belowground ecosystem functions similar to those that occur in diverse, natural ecosystems (Zak et al., 2003).

Soils represent one of the most biologically diverse habitats (Orgiazzi et al., 2016) but belowground species diversity does not follow a clear relationship with aboveground diversity (Shange et al., 2012). For instance, nitrogen (N) fertilization reduces aboveground diversity (Bobbink et al., 2010), but this is not necessarily reflected in belowground microbial communities (Ramirez et al., 2010). Diverse plant residue inputs alter belowground nutrient cycles carried out by microbes (Wardle, 2002); however, the links between aboveground diversity and belowground diversity may depend on the specific microbial process, such as methane oxidation (Levine et al., 2011) or denitrification (Powell et al., 2015).

Aboveground communities can shape the composition of soil microbial communities as well as alter the function of microbial processes, such as decomposition, which are common across these communities (Strickland et al., 2009). In cropping systems, management practices alter both microbial growth efficiency and mineralization of soil carbon (C) (Lee and Schmidt, 2014), as well as the fate of specific C substrates (Kallenbach et al., 2015). While aboveground plant diversity can enhance soil fertility (Zak et al., 2003), high fertility and microbial biomass in agricultural systems might not be directly tied to aboveground plant species *per se*, but rather to

number of crops in rotation and especially to presence of a cover crop (McDaniel et al., 2013). Over time, specific cropping systems can alter soil microbial diversity, but the reasons for this observation are not clear; for example, whether these changes are due to specific plant inputs or to rotational composition (Venter et al., 2016).

To help address this gap, we investigated the effect of increasing crop rotational complexity on bacterial community composition. We focus on a long term cropping system experiment that has received no external inputs (e.g. fertilizer or pest control agents), and varies only in the crop rotation, which includes a gradient from continuous summer annuals, corn (*Zea mays L.*) and soybean (*Glycine max.*), to summer annuals with a winter annual (wheat; *Triticum aestivum L.*), to inclusion of non-harvested cover crops (legumes and grasses) in rotation. In this cropping system experiment, increasing complexity of crops in rotation has resulted in greater crop productivity (Smith et al., 2008), altered microbial processing of soil substrates (McDaniel et al., 2016), increased soil C, enzyme activity, and soil aggregation (Tiemann et al., 2015), and has increased labile C pools and patterns in soil C oxidation (see chapter 2). We expect that increased crop productivity, diversity, and abundance of plant inputs entering the soil, and resulting changes in the spatial heterogeneity of the soil habitat, have also restructured soil bacterial communities. Along a cropping systems gradient from continuous grain monoculture to annual grains in rotation with cover crops, we expect that rotations with greater plant biomass inputs will restructure microbial community composition and increase bacterial diversity. Specifically, we expect that the effect of cover crops in rotation will have the greatest effect on bacterial community structure due to their strong effect on increasing mineralizable substrate (see chapter 2) and stimulating soil microbial biomass (McDaniel et al., 2013).

METHODS

Site description

We conducted our study at Michigan State University's Kellogg Biological Station (KBS) Long Term Ecological Research experiment located in Hickory Corners, Michigan (42° 24′ N, 85° 24′ W, elevation 288 m). KBS lies in SW Michigan, in the eastern part of the U.S. Corn Belt. Prior to 1988, the site had been conventionally managed for row crop agriculture (Robertson et al., 1997). Soils developed on glacial outwash are composed of Kalamazoo (Fine-loamy) and Oshtemo (Coarse-loamy) mixed, mesic, Typic Hapludalfs (FAO soil order: Luvisols). The climate is temperate, with approximately 1005 mm rainfall and an average snowfall of ~1.3 m. Mean annual temperature is 10.1° C (Robertson and Hamilton, 2015).

We used the KBS Biodiversity Gradient Experiment (http://lter.kbs.msu.edu/research/long-termexperiments/biodiversity-gradient/), which consists of a gradient of rotational complexity of harvested annual grains including corn, soybean, and wheat ranging from continuous monoculture (e.g., continuous corn, or continuous soybean) to various rotations that also include an unharvested overwintering cover crop of red clover (*Trifolium prantense L.*), crimson clover (*Trifolium incarnatum*), or annual ryegrass (*Lolium multiflorum Lam.*) either alone or in combinations. The study was established in 2000 and is composed of plant species treatments from bare ground, to 1, 2, 3, 4, 6 and 10 species in a randomized complete block experiment with four replicates. Treatment plots are 9 x 30 m and every phase of each crop rotation is represented every year. Since establishment, none of the treatments have received synthetic chemical inputs (i.e. no N additions of fertilizer or pest control agents) and weeds are controlled mechanically.

Soil sampling

For this study we selected six treatments (Table 3.1): continuous corn (cC) and continuous soy (cS), corn and soybean in rotation (CS), CS and wheat in rotation (CSW), CSW with inclusion of clover varieties as a winter cover crop (CSW1cov), and CSW with clovers and annual ryegrass in a cover crop mixture (CSW2cov). At initial sampling the rotations had been in place for ten years. Soil samples were collected after harvest each year: on November 18, 2010 and October 27, 2011. At each sampling, cores were taken along a crop row, offset by one meter from the central row in the plot, and at equal spacing along a 20 meter length, approximately four meters in from either edge of the plot. One-inch soil cores were taken to a depth of 10 cm. Two sub-samples, one at the furrow center and one from the middle of the furrow slope were taken from five locations, along a crop row. The 10 cores were sieved to 2 mm, homogenized, and maintained on ice during transport to the lab. In the laboratory, composited soil from each plot was subsampled for different analysis, and approximately fifty grams were frozen in Whirlpack ® bags and stored at -80° C.

Soil DNA extraction and PCR amplification

DNA was extracted from 0.25 g of each treatment soil using the MO-BIO PowerMag Soil DNA Isolation kit (MO BIO, Carlsbad, CA) on an Eppendorf epMotion 5075 TMX robot (Hamburg, Germany). The V4 region of the 16S rRNA gene was amplified using a dual-indexing approach (Kozich et al., 2013). PCR amplification and sequencing was performed using 1 µl of template DNA with the Illumina MiSeq platform, with the MiSeq Reagent Kit V2 500 cycles (Cat# MS-102-2003), according to the manufacturer's instructions except that Accuprime High Fidelity Taq (Life Cat # 12346094) was used instead of Accuprime Pfx supermix. Conditions for the reaction were 2 m at 95° C followed by 30 cycles at 95° C for 20 s,

55° C for 15 s and 72° C for 5 m, followed by 10 m at 72° C and then maintained at 4° C. Amplified products were visualized using an E-Gel 96 with SYBR Safe DNA Gel Stain, 2% (Life technologies cat# G7208-02) and normalized using Life technologies SequalPrep Normalization Plate Kit (cat # A10510-01) following the manufacturer's protocol. Samples were pooled and concentration determined using Kapa Biosystems Library Quantification kit for Illumina platforms (KapaBiosystems KK4824). The sizes of the amplicons in the library were determined using the Agilent Bioanalyzer High Sensitivity DNA analysis kit (cat# 5067-4626) so that the final library consists of equal molar amounts. Final library preparation and sequencing was carried out following the manufacture's protocol, MiSeq (part# 15039740 Rev. D), for 2nM or 4nM libraries. After DNA extraction, all sample preparation and DNA sequencing were carried out by the Host Microbiome Initiative at the University of Michigan.

Analysis of 16S bacterial communities

Sequences were sorted by sample and quality filtered using the mothur software (Schloss et al., 2009). Individual operational taxonomic units (OTUs) were classified using the SILVA database (Quast et al., 2013), and individual communities were sub-sampled to the number of sequences in the sample with the fewest amplicons for all further community analysis. Within mothur, richness was assessed using the Chao 1 estimator and diversity using Shannon and Inverse Simpson indices. Analysis of molecular variance (Amova) was used as a non-parametric method of testing for significant differences among populations. Finally, within mothur, differential abundance of taxa across treatments was assessed via Metastats, Lefse and Indicator species (McCune et al., 2002; Segata et al., 2011; White et al., 2009) to find consistent patterns in key taxa occurring in different rotations. Further community analysis was carried out using the Vegan package (Oksanen et al., 2013) in R (R Core Team, 2012), including non-metric

multidimensional scaling (using Bray-Curtis), analysis of similarity (ANOSIM) and perMANOVA (both with permutations=999), Mantel tests, and fitting of environmental variable onto bacterial communities.

RESULTS

Following quality filtering of 16S rDNA amplicons, the treatments were subsampled to 17,255 OTUs, the size of the smallest library across all treatments for both years. This resulted in an estimated coverage of no less than 94.8 ± 0.3 percent across all treatments (Table 3.2). Chao 1 OTU richness estimates ranged from a low of 3275 ± 120 in CSW2cov to 3459 ± 83 in CS with no significant difference across treatments. For estimates of diversity using Inverse Simpson's index, cS had the lowest value (299.8 ± 12.1), and cC the highest (324.1 ± 21.5), again with no significant difference or noticeable trend across crop rotation treatments.

Bacterial communities of treatments did differ (P=0.003) when variation in OTUs was transformed into a distance matrix (Bray-Curtis) with variance partitioned by treatment (controlling for block and year), and estimated using perMANOVA (Table 3.3), after checking for homogeneity for variance. The same analysis, when partitioned instead by the presence or absence of a cover crop, showed even stronger significance (P=0.001), but with higher residual error (Table 3.3). A similar result was found in ANOSIM, comparing similarities within grouped treatments (Table 3.3). Another distance-based estimate (Amova), showing pairwise comparisons, revealed no significant difference among treatments without cover crops, and the strongest divergence in communities between cC and CSW1cov (P=0.020), cC and CSW2cov (P=0.034), and CS and CSW1cov (P=0.0029) (Table 3.4). The patterns are clearly visible when

scaled and shown in two dimension using NMDS (Figure 3.1). The effect of year (and thus crop in rotation except for mS and mC) had no effect on this structure (Figure 3.2).

Using a matrix of soil nutrient cycling parameters along the rotational gradient (Figure 3.3, see also Chapter 2) we used a Mantel test for correlation between two separate years of environmental variables (transformed using Euclidean distances) and OTUs by rotation. For 2010, the correlation was not significant ($r=0.128$, $P=0.127$) and for 2011, this test was significant ($r=0.187$, $P=0.033$). Fitting vectors from soil environmental variables onto OTU distance matrix of crop rotations for 2010 showed significant goodness of fit (Table 3.5) with potentially mineralizable N (PMN; $P=0.008$), and C (PMC; $P=0.013$) as well as the enzyme N-acetyl-glucosaminidase (NAG) activity ($P=0.033$). For 2011, the stronger correlation of environmental and 16S communities matrices is evident in stronger relationships in fitting environmental factors: PMN ($P=0.009$), PMC ($P=0.001$), NAG ($P=0.017$), POXC (permanganate oxidizable C; $P=0.006$), a measure of labile C, as well as soil nitrate, ammonium, acid phosphatase activity and soil pH (Table 3.5).

Differences in the relative abundance in major bacteria phyla were not significant and showed no clear pattern among treatments or years (Figure 3.5). We ranked bacteria by the significance of their influence on distinguishing cover crop and non-cover crop treatments using a consensus of significance effects ($P\leq0.05$) between techniques in mothur: Lefse, Metastat, Indicator species. The 20 most influential OTUs representing cover and no-cover treatments represent approximately 11.0% of mean amplicons across all treatments (with coefficient of variation between individual treatments of 12%). The most influential OTUs in distinguishing cover crop rotation came from the *Proteobacteria*, while the most predominant OTUs distinguishing non-cover treatments were in the *Acidobacteria* (Figure 3.4, Table 3.6).

DISCUSSION

The legacy of crop rotation in this long term experiment significantly altered composition of soil bacterial, especially in rotations including cover crops which strongly influenced the community structure compared to treatments with no cover. In two successive years, the bacterial community did not shift based on the identity of the grain crop in rotation, and among treatments, the relative abundance of bacterial phyla was not significantly different. There was no evidence for differences in bacterial species richness or diversity based on rotation, as I had hypothesized. However, a clear pattern emerged in the taxa, which differentiated rotations with and without cover crops. Among unique OTUs, *Proteobacteria* taxa distinguished bacterial communities in cover crops while in rotations without cover crops taxa from the *Acidobacteria* dominated the bacterial community. The distinct taxa shaping bacterial community structure strongly correlated with soil ecosystem functions, especially for N and C mineralization between cover and no cover treatments.

While the linkages between aboveground plant diversity and belowground ecosystem functions are strong (Wardle, 2002), the effects on the diversity of soil organisms that carry out many of these functions is less clear. In agricultural systems, increasing crop rotation complexity has alternately been shown to increase bacterial diversity over the long term (Figuerola et al., 2015), to have no effect (Navarro-Noya et al., 2013), or to have a negative effect on diversity and richness (Yin et al., 2010). A recent meta-analysis assessing the effect of crop rotation on belowground microbial communities found evidence for a small increase in diversity with rotation, although findings differed in part due to methodological approach (Venter et al., 2016), with newer sequencing technologies revealing a decrease in diversity with increased crop rotation diversity. Similarly, controlled studies manipulating plant species

richness in grassland systems have demonstrated strong effects of richness on soil ecosystem function (Steinauer et al., 2015), and associated shifts in microbial community composition, but have found a negative correlation between plant species richness and soil bacterial diversity (Schlatter et al., 2015). Thus, the lack of any clear pattern in belowground bacterial diversity (Table 3.2), in spite of large differences in plant species richness and evenness, suggests that other factors shape belowground communities.

Agriculture has an strong effect on soil bacterial communities that persists for years after land is taken out of crop production (Buckley and Schmidt, 2001; Jangid et al., 2011). While soil bacterial communities may exhibit pronounced heterogeneity in the relative abundance of different phyla over very small spatial scales, at the ecosystem scale common structural patterns emerge, particularly linked to management (O'Brien et al., 2016). Seasonal row cropping of monocultures along with tillage practices may contribute to this ecosystem-scale community homogeneity. In this study, the relative abundance of major phyla did not differ across crop rotations (Figure 3.5), but bacterial community structure was significantly different by rotation, especially for treatments with cover crops (Table 3.3). However, this difference in bacterial communities by rotation, which remained stable regardless of most recent grain crop in rotation) without any change in bacterial diversity or richness may overlay a more dynamic and shifting bacterial community.

While a large segment of species composing estimates of bacterial diversity in soils may actually derive from dormant cells (Jones and Lennon, 2010), even in soils under decades-long monoculture cultivation, the introduction of new plant species can rapidly alter the soil bacterial community structure (Maul and Drinkwater, 2010). And, within ecosystems, the relative abundance of specific soil bacterial taxa shift with temporal and seasonal patterns (Buckley and

Schmidt, 2003). Furthermore, spatial heterogeneity created by soil aggregation is an important determinant of microbial community structure (Blackwood et al., 2006), and cover cropped treatments within this rotational gradient experiment have significantly higher soil aggregate formation than those without cover (Tiemann et al., 2015). Thus an interaction of short term plant species effects, seasonal effects, and spatial heterogeneity of the soil ecosystem may act to distinguish microbial community structure among rotations, especially for treatments with cover crops (Table 3.4).

However microbial communities in rotations with cover crops also appear to process soil C in a manner distinct from rotations without cover crops (Chapter 2), such that longer-term changes in substrate quality may be a more important factor shaping the microbial community structure and physiology (Frey et al., 2013) than the proximal influence of different plant species inputs (Maul and Drinkwater, 2010). Sampling for bacterial communities for each year of this study occurred post-harvest - November, 2010 after corn in and October, 2011 after soybean. In the corn year, cover cropped treatments were planted to overwintering species, with red clover interseeded into corn and rye planted post-harvest into CSW2cov (Table 3.1). In the soybean year cover cropped treatments were interseeded with crimson clover, which was terminated at soybean harvest and followed by wheat planting. Despite these short-term differences in the plant species present over the two years of sampling, the soil bacterial community structure was stable among similar treatments. That is, differences in community structure were driven not by the most recent crop species in rotation, but instead differed based on the long-term history of presence or absence of winter cover (Figure 3.1, Figure 3.2).

Increased cropping system diversity, and especially the use of cover crops, supports higher microbial biomass (McDaniel et al., 2013) and larger labile soil C and N pools (Chapter

2) readily accessible to microbes. Here, the bacterial taxa that most strongly distinguished treatments with cover crops from those without winter cover belonged to the *Proteobacteria* (Figure 3.4), while taxa that differentiated treatments without cover crops belonged to the *Acidobacteria*. Bacteria in these two phyla are among the most common in soil (Janssen, 2006) and their relative abundance often varies based on ecosystem type. For example forest ecosystems generally have a greater abundance of *Acidobacteria* taxa relative to agroecosystems, while the reverse is true for *Proteobacteria* (Jangid et al., 2008, 2011). Relative abundance of *Proteobacteria* tend to increase with N addition to soil (Ramirez et al., 2010) as many of these taxa appear to carry genes for numerous N cycling pathways (Nelson et al., 2015). These trends in the abundance of distinct taxa relative to available soil substrates align with patterns in nutrient cycling in treatments with and without cover crops (Chapter 2), but also point toward altered microbial metabolism and resource use across different rotations.

Distinct taxa in rotations with and without cover crops are an indication of the soil ecosystem function of these communities such as catabolic potential, and rates of C and N mineralization (McDaniel and Grandy, 2016). Rapid, inefficient growth is a characteristic of many *Proteobacteria* taxa (Roller et al., 2016), which respire more CO_2 per unit biomass production than slower-growing organisms (Roller and Schmidt, 2015). By contrast, the bacteria within the *Acidobacteria* are characterized by slow doubling times associated with more efficient growth in oligotrophic environments (Eichorst et al., 2007). In spite of this contrast in growth efficiency, microbial communities in croplands may have a greater overall bacterial growth efficiency (BGE) than those in adjacent deciduous forest soils (Lee and Schmidt, 2014). Yet it is notable that even though forest soils generally have greater soil C than adjacent cropland, within agricultural and managed grasslands, *Acidobacteria* abundance tends to increase as total soil C

declines (Schmidt and Waldron, 2015). Finally, N inputs to soil are known to lower the relative abundance of *Acidobacteria* (Cederlund et al., 2014), alter microbial respiration (Matulich and Martiny, 2014) and slow soil C decomposition (Ramirez et al., 2010). This may mean that while microbial communities are fundamentally altered by cropping, they respond differently to short term inputs of critical resources.

Rotations with cover crops have larger pools of labile C and N that rise and fall over the growing season in greater magnitude than rotations without cover crops (Chapter 2), and variation in these substrate fluxes are strongly correlated with the underlying bacterial community structure (Table 3.5). BGE in annual cropping systems also shifts dramatically over the growing season (Lee and Schmidt, 2014). Thus as substrate availability changes in annual cropping systems it may strongly reshape bacterial community structure and at different times favor taxa which process substrates with different efficiencies. Inclusion of cover crops in row crop rotations has been found to significantly alter the process of soil C decomposition (Kallenbach et al., 2015) leading to disproportionately higher soil C accumulation than rotations without cover (Syswerda and Robertson, 2014). While over the long term, increasing rotational complexity, with or without cover crops, had no effect on overall bacterial species richness or diversity, my results suggest that cover crops shift soil microbial communities along bacterial phylogenies with distinct physiologies which suggests a basis functional changes in C and N cycling under cover crops.

ACKNOWLEDGEMENTS

Support for this research was also provided by U.S. Department of Agriculture Soil Processes Program, grant # 2009-65107-05961, and the National Science Foundation Long-term Ecological Research Program (DEB 1027253) at the Kellogg Biological Station.

APPENDIX

Table 3.1 Crop rotations sampled on the KBS Biodiversity Gradient Experiment

Treatment	Rotation	Species / yr.	Species / rot.
cS	soy	1	1
cC	corn	1	1
CS	corn + soy	1	2
CSW	corn + soy + wheat	1-2	3
CSW1cov	corn + soy + wheat + clover	2-3	4
CSW2cov	corn + soy + wheat + clover + rye	3-4	5

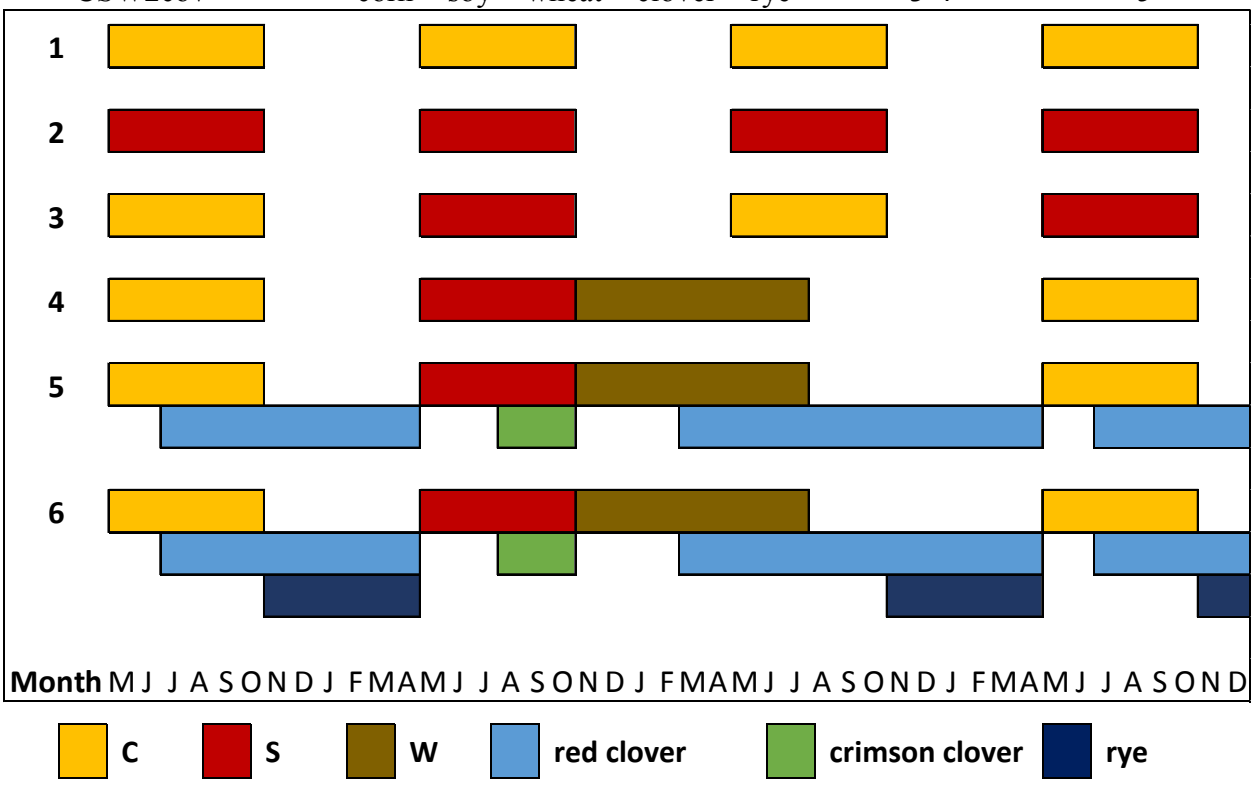


Table 3.2 Estimated population parameters 16S rDNA from rotations across the biodiversity gradient experiment (see table 3.1 for treatment acronyms), including mean ± se for coverage, estimated OTU richness (Chao1) and diversity (Inverse Simpsons).

Rotation	Coverage (%)	Chao 1	Inverse Simpsons
cS	95.1 ± 0.2	3279 ± 93	300 ± 12
cC	94.8 ± 0.3	3437 ± 123	315 ± 26
CS	94.8 ± 0.1	3458 ± 83	324 ± 21
CSW	94.8 ± 0.2	3408 ± 107	289 ± 12
CSW1cov	94.8 ± 0.2	3389 ± 117	301 ± 19
CSW2cov	95.1 ± 0.2	3275 ± 119	312 ± 16

Table 3.3 Multivariate analysis of 16S rDNA from the biodiversity gradient experiment using perMANOVA with two different factors, rotation and presence of a cover crop, showing the sources of variation, degrees of freedom (DF), sums of squares (SS) mean square (MS), F-statistic (F) and related coefficient of determination (R^2) and probability (Prob.). Analysis of similarities (ANOSIM) between grouped factors showing the correlation coefficient (R) and probability (Prob).

perMANOVA								ANOSIM	
Factor	Source	DF	SS	MS	F	R^2	Prob.	R	Prob.
Rotation	Rotation	5	0.55	0.1108	1.3	0.134	0.003	0.0928	0.003
	Residuals	42	3.58	0.0852	0.866				
	Total	47	4.13	1					
Cover	Cover	1	0.27	0.27	3.21	0.065	0.001	0.281	0.001
	Residuals	46	3.86	0.084	0.935				
	Total	47	4.13	1					

Table 3.4 Analysis of molecular variance (Amova) of 16S OTUs, with treatment-level (Overall) F-statistic (P) and table of pair-wise comparisons of individual treatments across the biodiversity gradient experiment. See table 3.1 for treatment acronyms.

Overall Among Populations 1.272 (0.008*)

	cS	cC	CS	CSW	CSW1cov	CSW2cov
cS	-	-	-	-	-	-
cC	0.870 (0.717)	-	-	-	-	-
CS	0.976 (0.474)	0.813 (0.836)	-	-	-	-
CSW	0.879 (0.689)	0.768 (0.918)	0.884 (0.685)	-	-	-
CSW1cov	1.710 (0.010)	1.661 (0.021)	2.329 (0.001)	1.681 (0.01)	-	-
CSW2cov	1.355 (0.065)	1.260 (0.122)	1.756 (0.001)	1.257 (0.092)	0.86 (0.768)	-

Table 3.5 Vector analysis of correlation between matrix of edaphic factors (Euclidean) and 16S rDNA (Bray-Curtis) for two years on the biodiversity gradient experiment. For each year the goodness of fit (r^2) and the significance, P, was calculated for soil parameters: inorganic N (NH_4^+-N and NO_3^--N), potentially mineralizable N (PMN) and C (PMC), permanganate oxidizable C (POXC), enzyme activities for beta-glucosidase (BG), peroxidase (PEROX) + phenol oxidase (PHENOX), N-acetyl-glucosaminidase (NAG) + tyrosine amino peptidase (TAP), acid phosphatase (PHOS).

	2010			2011		
Soil Factors	r^2	P		r^2	Pr(>r)	
PMN	0.37	0.008	**	0.38	0.009	**
PMC	0.36	0.013	*	0.62	0.001	***
NAG	0.28	0.033	*	0.35	0.017	*
POXC	0.16	0.166		0.41	0.006	**
NO_3^-	0.21	0.081	.	0.33	0.024	*
NH_4^+	0.18	0.113		0.25	0.050	*
PHOS	0.10	0.332		0.24	0.046	*
BG	0.20	0.111		0.17	0.155	
TAP	0.07	0.449		0.23	0.071	
PEROX	0.08	0.452		0.13	0.234	
PHENOX	0.02	0.825		0.00	0.961	.
pH	0.17	0.121		0.28	0.035	*

Table 3.6. The 20 the most influential OTUs ranked by their abundance (after normalizing and sub-setting across all treatments and replicates) and the significance ($P \leq 0.05$) of their influence on distinguishing cover crop and non-cover crop treatments.

Rotation	SILVA classification of OUT
Cover	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Sphingomonadales(100); Sphingomonadaceae(100);unclassified(100);
Cover	Bacteria(100);Bacteroidetes(100);Sphingobacteriia(100);Sphingobacteriales(100); Chitinophagaceae(100);Flavisolibacter(91);
Cover	Bacteria(100);Actinobacteria(100);Actinobacteria(100);Actinomycetales(100); Micrococcaceae(100);Arthrobacter(100);
Cover	Bacteria(100);Proteobacteria(100);Betaproteobacteria(100);unclassified(100); unclassified(100);unclassified(100);
Cover	Bacteria(100);Verrucomicrobia(100);Spartobacteria(100);unclassified(100); unclassified(100);unclassified(100);
Cover	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);unclassified(100); unclassified(100);unclassified(100);
Cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp16(100);Gp16(100); unclassified(100);unclassified(100);
Cover	Bacteria(100);Proteobacteria(100);Deltaproteobacteria(100);Myxococcales(100); unclassified(100);unclassified(100);
Cover	Bacteria(100);Actinobacteria(100);Actinobacteria(100);Actinomycetales(100); Micromonosporaceae(100);unclassified(84);
Cover	Bacteria(100);Proteobacteria(100);Deltaproteobacteria(100);Myxococcales(100); Myxococcaceae(79);Corallococcus(71);
Cover	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);unclassified(100); unclassified(100);unclassified(100);
Cover	Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100); unclassified(100);
Cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp3(100);Gp3(100);unclassified(100);un classified(100);
Cover	Bacteria(100);Verrucomicrobia(100);Spartobacteria(100);unclassified(100); unclassified(100);unclassified(100);
Cover	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100); Hyphomicrobiaceae(59);Rhodoplanes(59);
Cover	Bacteria(100);Proteobacteria(100);Deltaproteobacteria(100);Myxococcales(100); unclassified(100);unclassified(100);
Cover	Bacteria(100);candidate_division_WPS-1(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100);
Cover	Bacteria(100);Verrucomicrobia(100);Subdivision3(100);unclassified(100); unclassified(100);unclassified(100);
Cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp4(100);Gp4(100); unclassified(100);unclassified(100);
Cover	Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100); unclassified(100);

Table 3.5 (cont'd)

No cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp4(100);Gp4(100);unclassified(100); unclassified(100);
No cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp16(100);Gp16(100); unclassified(100);unclassified(100);
No cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp4(100);Gp4(100); unclassified(100);unclassified(100);
No cover	Bacteria(100);unclassified(100);unclassified(100);unclassified(100); unclassified(100);unclassified(100);
No cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp6(100);Gp6(100);unclassified(100); unclassified(100);
No cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp4(100);Gp4(100);unclassified(100); unclassified(100);
No cover	Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100); unclassified(100);
No cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp4(100);Gp4(100);unclassified(100); unclassified(100);
No cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp7(100);Gp7(100);unclassified(100); unclassified(100);
No cover	Bacteria(100);Gemmatimonadetes(100);Gemmatimonadetes(100); Gemmatimonadales(100);Gemmatimonadaceae(100);Gemmatimonas(100);
No cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp4(100);unclassified(100); unclassified(100);unclassified(100);
No cover	Bacteria(100);Verrucomicrobia(100);Spartobacteria(100);unclassified(100); unclassified(100);unclassified(100);
No cover	Bacteria(100);Proteobacteria(100);Betaproteobacteria(100);unclassified(100); unclassified(100);unclassified(100);
No cover	Bacteria(100);Nitrospirae(100);Nitrospira(100);Nitrospirales(100);Nitrospiraceae(100); Nitrospira(100);
No cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp4(100);Gp4(100);unclassified(100); unclassified(100);
No cover	Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100); unclassified(100);
No cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp6(100);Gp6(100);unclassified(100); unclassified(100);
No cover	Bacteria(100);unclassified(100);unclassified(100);unclassified(100);unclassified(100); unclassified(100);
No cover	Bacteria(100);Actinobacteria(100);Actinobacteria(100);Actinomycetales(100);Geoderm atophilaceae(100);Geodermatophilus(65);
No cover	Bacteria(100);Acidobacteria(100);Acidobacteria_Gp17(100);Gp17(100); unclassified(100);unclassified(100);

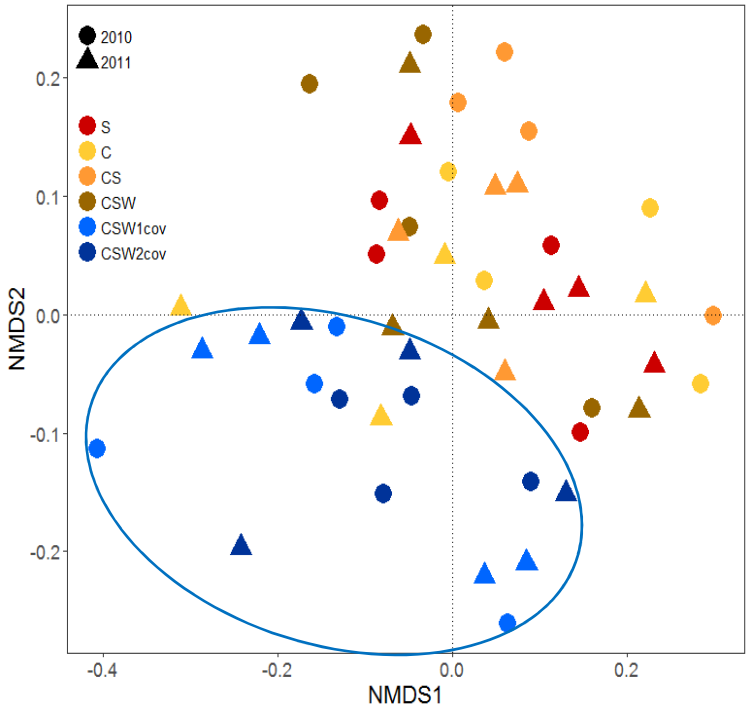


Figure 3.1 Non-metric multidimensional scaling (NMDS) plot of 16S rDNA of KBS biodiversity gradient for combined two years of study with rotations in different colors, and years in different shapes (Stress = 0.1681). Blue ellipse encircles treatments with cover crops. See table 3.1 for treatment acronyms.

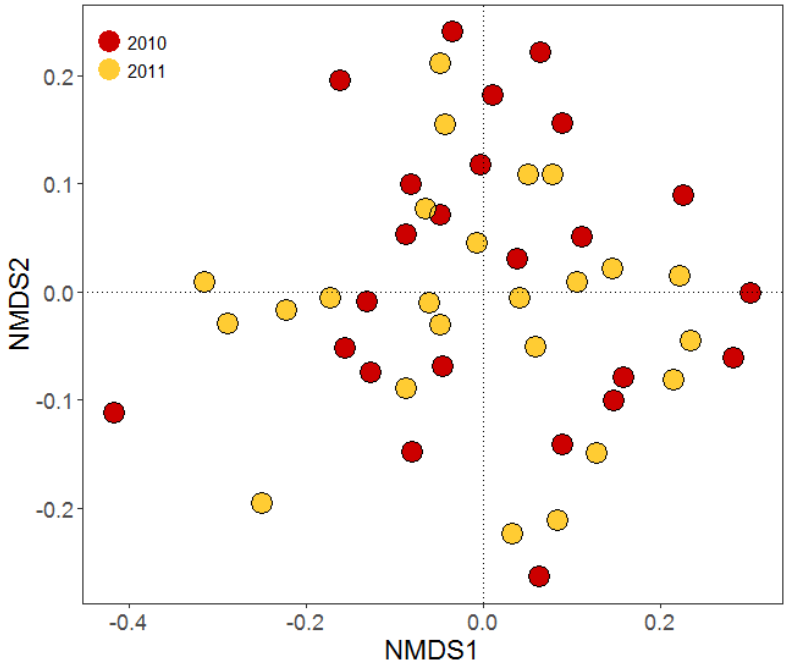


Figure 3.2 Non-metric multidimensional scaling (NMDS) plot of 16S rDNA of KBS biodiversity gradient with all rotations shown by sampling year. (Stress = 0.1681).

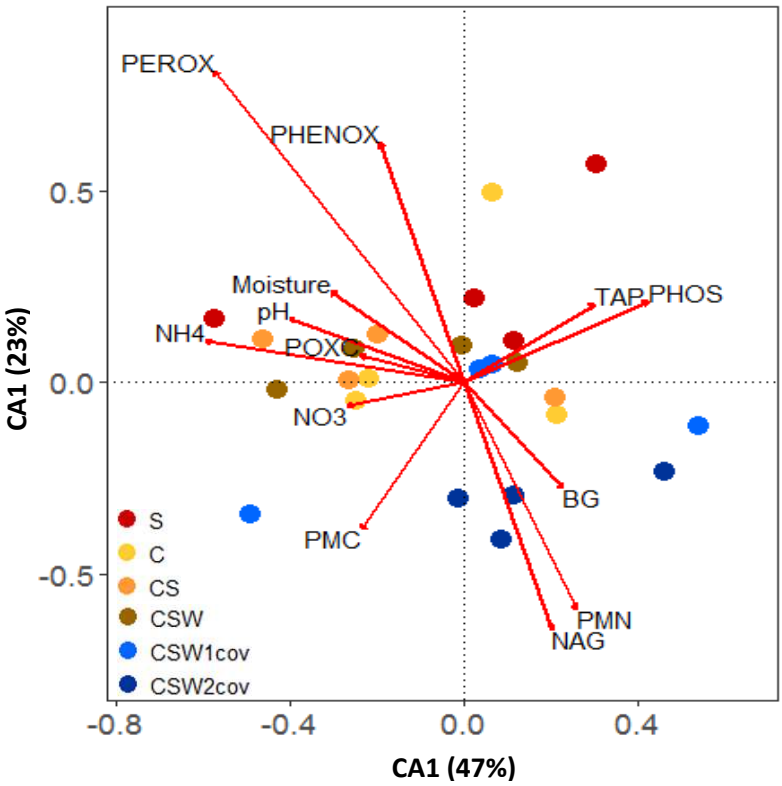


Figure 3.3 Canonical correspondence analysis plot of KBS biodiversity gradient for combined two years of study with differences in rotations constrained to measured soil parameters and the two axes representing the greatest explained variation (on a percentage basis) . Figures are from cumulative measures over the season for all parameters except pH (which represents a mean value). Other parameters are Moisture (soil moisture), inorganic N (NH_4^+-N and NO_3^--N), potentially mineralizable N (PMN) and C (PMC), permanganate oxidizable C (POXC), enzyme activities for beta-glucosidase (BG), peroxidase (PEROX) + phenol oxidase (PHENOX), N-acetyl-glucosaminidase (NAG) + tyrosine amino peptidase (TAP), acid phosphatase (PHOS). All ordinations were created with a correlational matrix to give equal weights to all soil parameters. See table 3.1 for treatment acronyms.

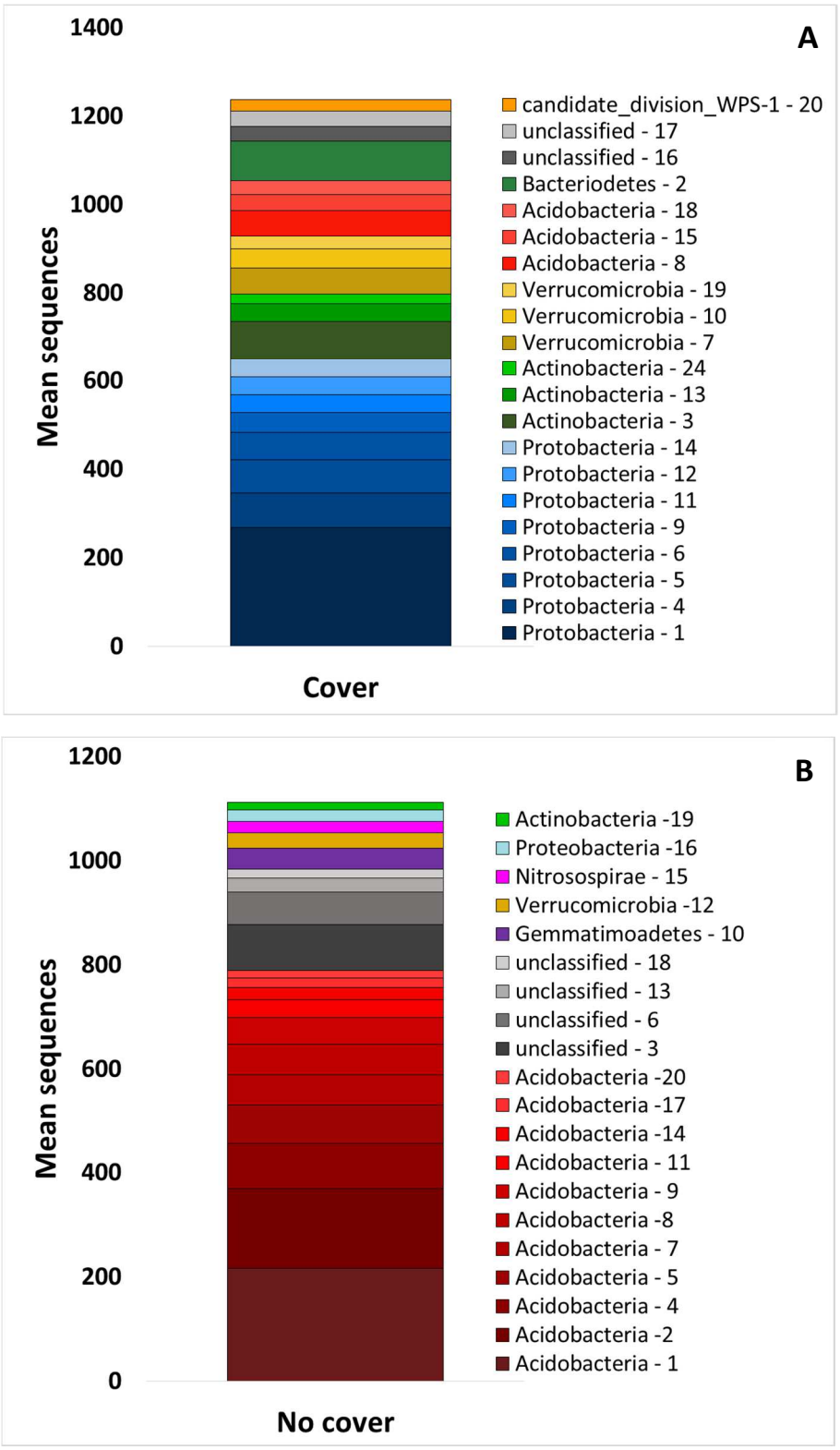


Figure 3.4 Phyla of 20 most abundant OTUs, significantly ($P \leq 0.05$) related to treatments with cover crops (A) or with no cover crops (B). Values are mean sequences across treatments of cover vs. no cover from normalized, subsampled 16S rDNA libraries

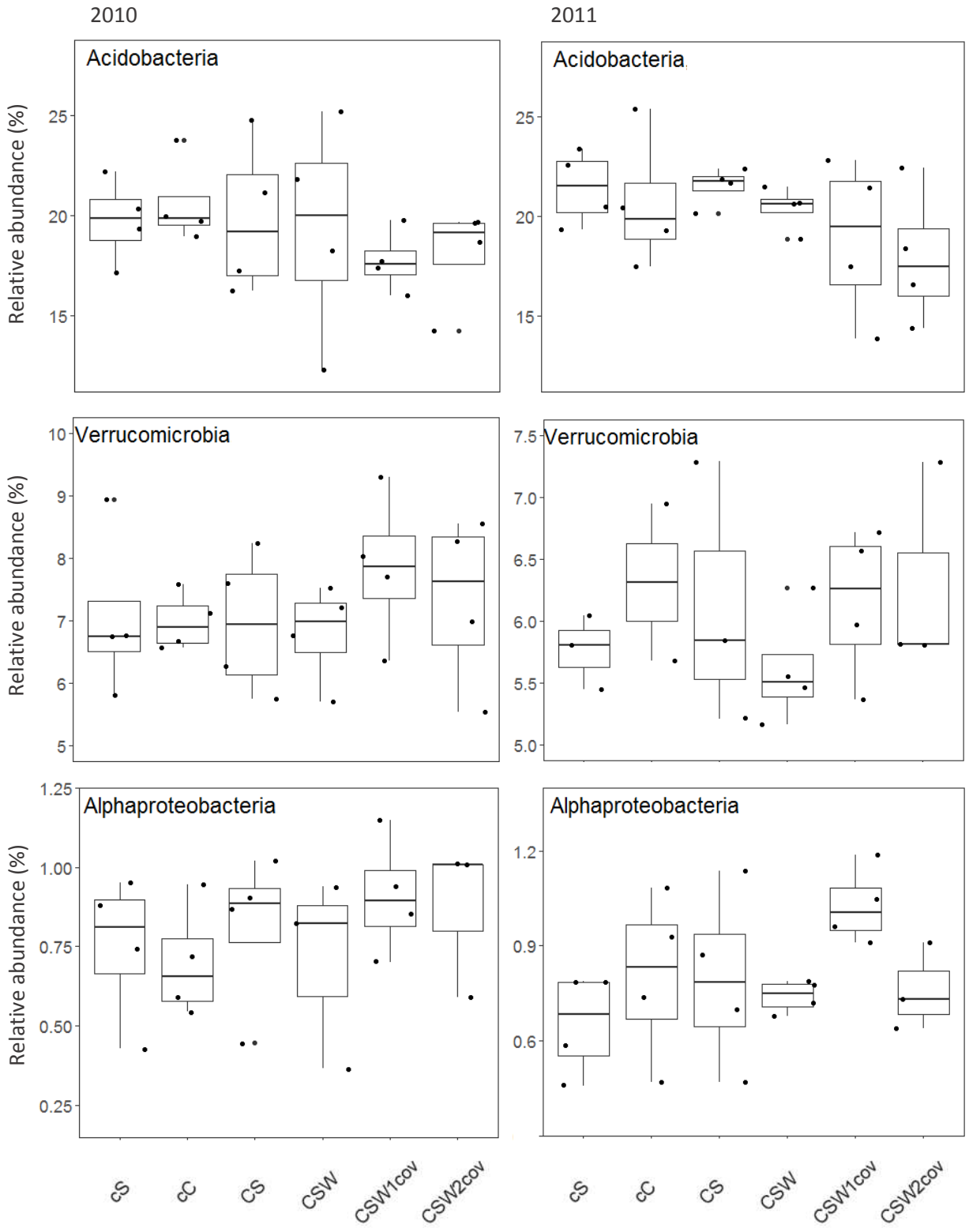


Figure 3.5 Relative abundance, after subsampling OTUs across rotations in the biodiversity experiment gradient of bacteria phyla (Acidobacteria, Verrucomicrobia) and class (Alphaproteobacteria) for two years. Box and whisker plots are shown with points from individual treatments (n=4). See table 3.1 for treatment acronyms.

REFERENCES

REFERENCES

Balmford, A., Green, R., Phalan, B., 2012. What conservationists need to know about farming. Proceedings of the Royal Society B: Biological Sciences 279, 2714–2724. doi:10.1098/rspb.2012.0515

Blackwood, C.B., Dell, C.J., Smucker, A.J.M., Paul, E.A., 2006. Eubacterial communities in different soil macroaggregate environments and cropping systems. Soil Biology and Biochemistry 38, 720–728. doi:10.1016/j.soilbio.2005.07.006

Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological Applications 20, 30–59.

Buckley, D.H., Schmidt, T.M., 2003. Diversity and dynamics of microbial communities in soils from agro-ecosystems. Environmental Microbiology 5, 441–452. doi:10.1046/j.1462-2920.2003.00404.x

Buckley, D.H., Schmidt, T.M., 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microb Ecol 42, 11–21. doi:10.1007/s002480000108

Cederlund, H., Wessén, E., Enwall, K., Jones, C.M., Juhanson, J., Pell, M., Philippot, L., Hallin, S., 2014. Soil carbon quality and nitrogen fertilization structure bacterial communities with predictable responses of major bacterial phyla. Applied Soil Ecology 84, 62–68. doi:10.1016/j.apsoil.2014.06.003

Eichorst, S.A., Breznak, J.A., Schmidt, T.M., 2007. Isolation and Characterization of Soil Bacteria That Define Terriglobus gen. nov., in the Phylum Acidobacteria. Appl. Environ. Microbiol. 73, 2708–2717. doi:10.1128/AEM.02140-06

Figuerola, E.L.M., Guerrero, L.D., Türkowsky, D., Wall, L.G., Erijman, L., 2015. Crop monoculture rather than agriculture reduces the spatial turnover of soil bacterial communities at a regional scale. Environ Microbiol 17, 678–688. doi:10.1111/1462-2920.12497

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O/'Connell, C., Ray, D.K., West, P.C., Balzer, C., Bennett, E.M., Carpenter, S.R., Hill, J., Monfreda, C., Polasky, S., Rockstrom, J., Sheehan, J., Siebert, S., Tilman, D., Zaks, D.P.M., 2011. Solutions for a cultivated planet. Nature 478, 337–342. doi:10.1038/nature10452

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

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., Wardle, D.A., 2005. Effects of Biodiversity on Ecosystem Functioning: A Consensus of Current Knowledge. Ecological Monographs 75, 3–35.

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

Jangid, K., Williams, M.A., Franzluebbers, A.J., Schmidt, T.M., Coleman, D.C., Whitman, W.B., 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biology and Biochemistry 43, 2184–2193. doi:10.1016/j.soilbio.2011.06.022

Janssen, P.H., 2006. Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S rRNA Genes. Appl. Environ. Microbiol. 72, 1719–1728. doi:10.1128/AEM.72.3.1719-1728.2006

Jones, S.E., Lennon, J.T., 2010. Dormancy contributes to the maintenance of microbial diversity. PNAS 107, 5881–5886. doi:10.1073/pnas.0912765107

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

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and environmental microbiology 79, 5112–5120.

Lee, Z.M., Schmidt, T.M., 2014. Bacterial growth efficiency varies in soils under different land management practices. Soil Biology and Biochemistry 69, 282–290. doi:10.1016/j.soilbio.2013.11.012

Levine, U.Y., Teal, T.K., Robertson, G.P., Schmidt, T.M., 2011. Agriculture's impact on microbial diversity and associated fluxes of carbon dioxide and methane. ISME J 5, 1683–1691.

Matulich, K.L., Martiny, J.B.H., 2014. Microbial composition alters the response of litter decomposition to environmental change. Ecology 96, 154–163. doi:10.1890/14-0357.1

Maul, J., Drinkwater, L., 2010. Short-term plant species impact on microbial community structure in soils with long-term agricultural history. Plant Soil 330, 369–382. doi:10.1007/s11104-009-0211-y

McCune, B., Grace, J.B., Urban, D.L., 2002. Analysis of ecological communities. MjM software design Gleneden Beach.

McDaniel, M.D., Grandy, A.S., 2016. Soil microbial biomass and function are altered by 12 years of crop rotation. SOIL Discussions 1–39. doi:10.5194/soil-2016-39

McDaniel, M.D., Grandy, A.S., Tiemann, L.K., Weintraub, M.N., 2016. Eleven years of crop diversification alters decomposition dynamics of litter mixtures incubated with soil. Ecosphere 7, n/a-n/a. doi:10.1002/ecs2.1426

McDaniel, M.D., Tiemann, L.K., Grandy, A.S., 2013. Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. Ecological Applications 24, 560–570. doi:10.1890/13-0616.1

Navarro-Noya, Y.E., Gómez-Acata, S., Montoya-Ciriaco, N., Rojas-Valdez, A., Suárez-Arriaga, M.C., Valenzuela-Encinas, C., Jiménez-Bueno, N., Verhulst, N., Govaerts, B., Dendooven, L., 2013. Relative impacts of tillage, residue management and crop-rotation on soil bacterial communities in a semi-arid agroecosystem. Soil Biology and Biochemistry 65, 86–95. doi:10.1016/j.soilbio.2013.05.009

Nelson, M.B., Berlemont, R., Martiny, A.C., Martiny, J.B.H., 2015. Nitrogen Cycling Potential of a Grassland Litter Microbial Community. Appl. Environ. Microbiol. 81, 7012–7022. doi:10.1128/AEM.02222-15

O'Brien, S.L., Gibbons, S.M., Owens, S.M., Hampton-Marcell, J., Johnston, E.R., Jastrow, J.D., Gilbert, J.A., Meyer, F., Antonopoulos, D.A., 2016. Spatial scale drives patterns in soil bacterial diversity. Environ Microbiol 18, 2039–2051. doi:10.1111/1462-2920.13231

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., others, 2013. Vegan: Community Ecology Package.

Orgiazzi, A., Bardgett, R.D., Barrios, E., Behan-Pelletier, V., Briones, M.J.I., Chotte, J.-L., De Deyn, G.B., Eggleton, P., Fierer, N., Fraser, T., Hedlund, K., Jeffery, S., Johnson, N.C., Jones, A., Kandeler, E., Kaneko, N., Lavelle, P., Lemanceau, P., Miko, L., Montanarella, L., Moreira, F.M.S., Ramirez, K.S., Scheu, S., Singh, B.K., Six, J., van der Putten, W.H., Wall, D.H., 2016. Global soil biodiversity atlas. Publications Office of the European Union, Luxembourg.

Powell, J.R., Welsh, A., Hallin, S., 2015. Microbial functional diversity enhances predictive models linking environmental parameters to ecosystem properties. Ecology 96, 1985–1993. doi:10.1890/14-1127.1

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucl. Acids Res. 41, D590–D596. doi:10.1093/nar/gks1219

R Core Team, 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A., Fierer, N., 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. Ecology 91, 3463–3470. doi:10.1890/10-0426.1

Robertson, G.P., Hamilton, S.K., 2015. Long-term ecological research in agricultural landscapes at the Kellogg Biological Station LTER site: conceptual and experimental framework, in: The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability. Oxford University Press, New York, New York, USA, pp. 1–32.

Roller, B.R., Schmidt, T.M., 2015. The physiology and ecological implications of efficient growth. ISME J 9, 1481–1487. doi:10.1038/ismej.2014.235

Roller, B.R.K., Stoddard, S.F., Schmidt, T.M., 2016. Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. Nature Microbiology 1, 16160. doi:10.1038/nmicrobiol.2016.160

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

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Horn, D.J.V., Weber, C.F., 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl. Environ. Microbiol. 75, 7537–7541. doi:10.1128/AEM.01541-09

Schmidt, T.M., Waldron, C., 2015. Microbial Diversity in Soil of Agricultural Landscapes and Its Relation to Ecosystem Functions, in: The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability. Oxford University Press, USA.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biology 12, R60. doi:10.1186/gb-2011-12-6-r60

Shange, R.S., Ankumah, R.O., Ibekwe, A.M., Zabawa, R., Dowd, S.E., 2012. Distinct Soil Bacterial Communities Revealed under a Diversely Managed Agroecosystem. PLoS ONE 7, e40338. doi:10.1371/journal.pone.0040338

Smith, R., Gross, K., Robertson, G., 2008. Effects of Crop Diversity on Agroecosystem Function: Crop Yield Response. Ecosystems 11, 355-366–366.

Steinauer, K., Tilman, D., Wragg, P.D., Cesarz, S., Cowles, J.M., Pritsch, K., Reich, P.B., Weisser, W.W., Eisenhauer, N., 2015. Plant diversity effects on soil microbial functions and enzymes are stronger than warming in a grassland experiment. Ecology 96, 99–112. doi:10.1890/14-0088.1

Strickland, M.S., Lauber, C., Fierer, N., Bradford, M.A., 2009. Testing the functional significance of microbial community composition. Ecology 90, 441–451. doi:10.1890/08-0296.1

Syswerda, S.P., Robertson, G.P., 2014. Ecosystem services along a management gradient in Michigan (USA) cropping systems. Agriculture, Ecosystems & Environment 189, 28–35. doi:10.1016/j.agee.2014.03.006

Tiemann, L.K., Grandy, A.S., Atkinson, E.E., Marin-Spiotta, E., McDaniel, M.D., 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. Ecol Lett 18, 761–771. doi:10.1111/ele.12453

Venter, Z.S., Jacobs, K., Hawkins, H.-J., 2016. The impact of crop rotation on soil microbial diversity: A meta-analysis. Pedobiologia.

Wardle, D.A., 2002. Communities and ecosystems: linking the aboveground and belowground components. Princeton University Press.

West, P.C., Gerber, J.S., Engstrom, P.M., Mueller, N.D., Brauman, K.A., Carlson, K.M., Cassidy, E.S., Johnston, M., MacDonald, G.K., Ray, D.K., others, 2014. Leverage points for improving global food security and the environment. Science 345, 325–328.

White, J.R., Nagarajan, N., Pop, M., 2009. Statistical Methods for Detecting Differentially Abundant Features in Clinical Metagenomic Samples. PLOS Computational Biology 5, e1000352. doi:10.1371/journal.pcbi.1000352

Yin, C., Jones, K.L., Peterson, D.E., Garrett, K.A., Hulbert, S.H., Paulitz, T.C., 2010. Members of soil bacterial communities sensitive to tillage and crop rotation. Soil Biology and Biochemistry 42, 2111–2118. doi:10.1016/j.soilbio.2010.08.006

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant Diversity, Soil Microbial Communities, and Ecosystem Function: Are There Any Links? Ecology 84, 2042–2050. doi:10.1890/02-0433

CHAPTER 4: LEGUME COVER CROPS ALTER MICROBIAL CONTROLS ON SOIL DENITRIFICATION RATES AND EFFICIENCY

ABSTRACT

Negative consequences of intensive inputs into agriculture, such as disruption of the global nitrogen (N) cycle, have led to calls to improve ecosystem regulation of nutrients by increasing diversity in cropping systems. Here we examine a long term row cropping experiment which has received no external inputs except for biological N fixation, to see how increasing crop rotational complexity, and in particular inclusion of cover crops, influences soil nitrous oxide (N_2O) emissions. Across a range of rotations from continuous monoculture, to rotations of annual grains, to inclusion of overwintering cover crops, we measured N_2O flux rates, soil N pools, denitrification potential, and a gene for the nitrite reductase (*nirK*) pathway leading to N_2O. Mineral N values were highest in rotations with cover crops and in continuous soybean monoculture. However potentially mineralizable N (PMN) was highest in rotations with cover crops and an adjacent fallow system, ranging between 8.6 and 9.0 μg NH_4^+-N g^{-1} which was approximately twice the concentration of all other rotations without cover crops. Soil mineral N and PMN were not as strong predictors of N_2O as the presence of a legume cover crop – these rotations had the highest mean flux of 13.2 g N_2O-N ha^{-1} day^{-1} in year one and 5.2 g N_2O-N ha^{-1} day^{-1} in year two which was 3-4 times higher than all other rotations. Denitrification in rotations with winter cover was also distinct. The ratio of N_2O produced out of total denitrification (rN_2O) under cover crops was between 0.51-0.59, and significantly lower when compared to rotations without cover which ranged from 0.7-0.83. In addition, rotations with cover crops had a significantly greater abundance of nitrite reductase genes (*nirK*) derived from ammonia oxidizing bacteria, suggesting greater potential for nitrifier denitrification. My results show that neither high soil mineral N nor potential mineralization rates were sufficient to predict N_2O flux,

but rather the presence of legume cover crops in rotation combined with a shift in the microbial community were most closely related to rates of denitrification.

INTRODUCTION

The intensification of row crop agricultural production into only a few annual grain crop species dependent on high inputs of mineral fertilizers has altered how soil and plants regulate nutrient cycling (Drinkwater and Snapp, 2007; Vandermeer et al., 1998). The alteration of the global nitrogen (N) cycle through agriculture, especially with anthropogenic additions of industrially-fixed N now exceeding natural (e.g. microbially-fixed) sources of N into soils (Galloway et al., 2004), has far-reaching downstream impacts and environmental consequences (Robertson and Vitousek, 2009). Agriculture is the leading source of increasing rates of atmospheric nitrous oxide (N_2O), a potent greenhouse gas with 300 times the global warming potential per molecule than carbon dioxide (IPCC, 2013). N_2O production is largely a naturally occurring process in soil, which has intensified due to land use change and N inputs to agriculture (Shcherbak et al., 2014), highlighting the need to both understand and mitigate N_2O from agriculture (Robertson and Vitousek, 2009). One approach to tightening nutrient cycling in soil is to restore plant diversity to the landscape and reduce intensive fertilizer inputs (Liebman and Davis, 2000). However, in row crops, the effect of increasing plant diversity on N_2O emissions, while maintaining productive grain-based agroecosystems, is not well understood.

A broad range of microorganisms are responsible for soil N transformations and N_2O production largely through a series of oxidation-reduction reactions (Leininger et al., 2006; Robertson and Groffman, 2015; Shoun et al., 1992). Nitrification is the critical pathway linking mineral N transformations of ammonium (NH_4^+) to nitrite (NO_2^-) and then to nitrate (NO_3^-)

(Robertson and Groffman, 2015). In the first step, autotrophic ammonium oxidizing bacteria (AOB) oxidize NH_4^+ as their principle source of energy, however under low oxygen conditions or excess NO_2^-, AOB will reduce the latter to N_2O (Kool et al., 2011). Denitrification is an anaerobic process in which heterotrophic microbes respire C substrates via the serial reduction of NO_3^- back to nitrite NO_2^-, nitric oxide (NO^-), N_2O and finally to N_2 (Zumft and Cárdenas, 1979). This process is crucial to the global N cycle as it returns reactive N back to the atmosphere (Gruber and Galloway, 2008), though in the process denitrification also releases N_2O, which is exacerbated in agroecosystems (Gelfand et al., 2016; Robertson et al., 2000).

In nature, the relative contribution of nitrification and denitrification to N_2O production is difficult to ascertain, and while each is favored under different conditions these distinct processes also share critical components. Unlike nitrification, denitrification is an anoxic process and requires a C substrate, the lack of which can result in incomplete denitrification and higher potential N_2O flux (Groffman et al., 2006). In addition, denitrification potential, or the rate of N_2O production relative to N_2, is distinct in agricultural soils in part due to the structure of the microbial community and the enzymes expressed in the denitrification pathway (Cavigelli and Robertson, 2000). The genes in this pathway, such as dissimilatory nitrite reductase (*nirK*), are shared across many bacterial lineages (Jones et al., 2008), including AOBs that carry out nitrification (Casciotti and Ward, 2001), thus offering one approach to distinguish between processes which produce N_2O in soil.

Numerous environmental and management factors influencing the N cycle can directly and indirectly influence N_2O emissions. Within agroecosystems N_2O flux can be affected by crop type, fertilization type and rate, tillage practice, and soil organic C (Gelfand et al., 2016; Smith et al., 2011; Stehfest and Bouwman, 2006). For example, tillage leads to soil N

mineralization and a pulse in soil N_2O (Grandy and Robertson, 2007). Plant communities have a strong effect on N_2O production, with perennial systems having far lower emissions than agroecosystems (Gelfand et al., 2016; Oates et al., 2016). This follows findings in natural ecosystems where plant diversity exerts a strong influence over the efficiency of soil nutrient cycles (Tilman et al., 2001) and the N cycle in particular (Zak et al., 2003). And while plant species diversity can increase soil fertility (Dybzinski et al., 2008), suggesting that increased diversity in agriculture can offset high external inputs of mineral N (Blesh and Drinkwater, 2013), implementing diverse row crop systems that achieve high N use efficiency remains a challenge (Robertson and Vitousek, 2009). Increasing the plant diversity of crop rotations (e.g. by introducing legume cover crops) to enhance soil nutrient cycling and ecosystem processes may also alter the many factors that control N_2O emissions.

In this study we examine the effect of increasing rotational diversity in row crops on soil N_2O production in a long term cropping system that has received no external inputs (e.g. fertilizer or pesticide) for over ten years. Plant composition ranges from continuous monoculture to rotated annual grains with and without winter cover. Specifically we hypothesize that greater abundance of legumes in rotation will increase nitrate availability and lead to greater N_2O flux. We expect this effect to be stronger in rotations with legume species, and smaller in rotations that also included small grains, which would increase synchrony of mineral N uptake and reduce N_2O flux. In addition we predict that the long term effect of different crop rotations will alter the composition of genes in the denitrification pathway, specifically *nirK*. Finally, we hypothesize that more complex rotations, with greater frequency of legumes and small grains, will lead to more complete denitrification (measured as DEA), due to greater coupling of C and N cycles, and abundance of labile C and N forms.

METHODS

Site descriptions and experimental layout

We conducted our study at Michigan State University's Kellogg Biological Station (KBS) Long Term Ecological Research site in Hickory Corners, Michigan (42° 24′ N, 85° 24′ W, elevation 288 m). KBS lies in SW Michigan, in the eastern part of the U.S. Corn Belt. Prior to 1988, the site had been conventionally managed for row crop agriculture (Robertson et al., 1997). Soils developed on glacial outwash and are composed of Kalamazoo (Fine-loamy) and Oshtemo (Coarse-loamy) mixed, mesic, Typic Hapludalfs (FAO soil order: Luvisols). The climate is temperate, with approximately 1005 mm rainfall and an average snowfall of ~1.3 m. Mean annual temperature is 10.1° C (Robertson and Hamilton, 2015).

This work was undertaken on the KBS Biodiversity Gradient Experiment (http://lter.kbs.msu.edu/research/long-termexperiments/biodiversity-gradient/) which consists of a gradient of rotational complexity of harvested annual grains including corn, soybean, and wheat ranging from continuous monoculture (e.g., continuous corn, or continuous soybean) to various rotations that also include an non-harvested overwintering cover crop of red clover (*Trifolium prantense L.*), crimson clover (*Trifolium incarnatum*), or annual ryegrass (*Lolium multiflorum Lam.*) either alone or in combinations. The study was established in 2000 and is composed of plant species treatments from bare ground, 1, 2, 3,4, 6 and 10 species, in a randomized complete block experiment with four replicates within 9 x 30 m treatment plots and with every phase of each crop rotation represented every year. Since establishment, none of the treatments have received synthetic chemical inputs (i.e. no N fertilizer or pest control agents) and weeds are controlled mechanically.

For this study we selected seven treatments (Fig. 4.1): continuous corn (cC) and continuous soy (cS), corn and soybean in rotation (CS), CS and wheat in rotation (CSW), CSW with inclusion of clover varieties (CSW1cov), CSW with clovers and annual ryegrass (CSW2cov), and a fallow plot tilled once each spring (Fspring) but otherwise allowed to return to early secondary succession. Sampling occurred over five month periods during two growing seasons, beginning in 2010 at planting, with most treatments in corn (except for cS and Fspring) until after soybean harvest in 2011 (except for cC and Fspring).

Soil Sampling

Over each study year I sampled all treatments and blocks for soil C and N parameters, and enzyme activity. I collected ten cores to 10 cm soil depth from five locations along a central row, alternating cores from within and between crop rows. Cores were composited by plot and placed on ice for transport to the lab. Within 24 hours of collection soils were sieved to < 2mm, mixed, and weighed for soil moisture, enzymes, and mineral N analyses.

Soil carbon and nitrogen

A portion of air-dried soils was finely ground for determination of total SOC and total soil organic nitrogen (SON) by dry combustion on a Costech ECS 4010 CHNSO Analyzer (Costech Analytical Technologies, Valencia, CA). Air-dried soil was used to determine labile soil C as permanganate oxidizable carbon (POXC) according to Weil et al. (2003). Duplicate 2.5 g samples of dried soil were mixed with buffered 0.02 M KMnO4 solution in 50ml conical tubes, shaken at 120 rpm for two minutes and allowed to settle for eight minutes. From this reaction, 0.5 mL of supernatant were diluted with 49.5 mL of deionized water. The degree of oxidation was measured colorimetrically at 550 nm on a Fisher Scientific Thermo Multiskan microplate

reader (Waltham, MA.) and standardized to a series of known $KMnO_4$ standards. Water-filled pore space (WFPS) was determined for air-died samples (Haney and Haney, 2010) and potentially mineralizable carbon (PMC) was adapted from Franzluebbers et al. (2000) and Haney (2008). Deionized water was added to 15 g of air-dried soil to 50% WFPS in canning jars with lids fitted with airtight, rubber septa. Lids were placed loosely on the jars and soils were incubated in the dark at 25° C. At 24-hours, jars were removed and soil respiration determined using a LI-COR LI-820 infrared gas analyzer (LI-COR Biosciences, Lincoln, NE): jars were sealed and a 0.5 mL of headspace gas was immediately removed and injected into the gas analyzer, followed by three additional measurements over approximately 90 min. PMC was calculated as CO_2 flux per gram of incubated soil.

Soil mineral N was extracted from fresh soil within 24 hours of sampling. Triplicate 10.0 g subsamples of sieved soil were extracted with 1M KCl for one hour on a rotary shaker (120 rpm). Samples were filtered and stored at -20 °C until analysis for ammonium (NH_4^+) and nitrate (NO_3^-). Potentially mineralizable nitrogen (PMN) was assessed anaerobically according to Drinkwater et al. (1996). Briefly, 10 mL of deionized water was added to triplicate 8 g fresh, sieved soil in conical tubes. Dinitrogen gas was used to replace tube headspace air and bubbled into the slurry for one minute prior to sealing with butyl rubber stoppers. Sealed tubes were incubated at 25 °C for seven days. After seven days the stoppers were removed, buffer was added to bring the slurry to 1M KCl, and samples were shaken, filtered, and stored in the same manner as fresh samples. Concentrations of NH_4^+ and NO_3^- were determined colorimetrically on a 96-well plate-reader. Soil NO_3^- was determined according to Doane and Horwáth (2003) and read at 540 nm, and soil NH_4^+ concentration determined at 630 nm (Sinsabaugh et al., 2000).

After incubation and extraction, PMN was determined based on the concentration of NH_4^+ incubated soil minus NH_4^+ from initial soil extraction of the same soil.

Soil Enzymes

To analyze soil enzymes, soil pH was determined using 10 g fresh soil mixed with 20 mL of deionized water, which was allowed to equilibrate for one hour with occasional stirring. Following this determination, the pH of a 50 mM sodium acetate buffer was adjusted to the pH of treatment soils. Approximately half of 100 mL of sodium acetate buffer was added to 1 g of treatment soil, and homogenized vigorously in a blender for exactly one minute. The rest of the buffer was used to rinse remaining soil contents from the blender into the slurry and then stored at 4° C until analysis (within 1-2 hours). In soil slurries we measured enzymatic potentials for β-1-4-N-acetyl-glucosaminidase (NAG) and tyrosine amino peptidase (TAP). At initiation of enzyme assays, soil slurries were constantly stirred and the suspension was pipetted into 96-well plates. Hydrolytic enzyme activity was monitored using substrate specific polymers containing fluorescent labels (either 4-methylumbelliferone or methylcoumarin). Enzymes were incubated in the dark at 15° C. At 4-6 hours, hydrolytic enzyme reactions were stopped by adding 10 µL, 1M sodium hydroxide, and plates were read on a Fisher Scientific Thermo Fluoroskan Ascent™ microplate fluorometer (Waltham, MA). Background absorbance-emission or fluorescence was accounted for with controls for buffer, buffer + soil, buffer + substrate.

In situ *nitrous oxide*

At approximately 2-week intervals soil N_2O fluxes were measured using static 25 cm-diameter chambers placed *in situ* in each treatment. The soil surface inside the chamber was maintained free of living plant material. PVC chambers were inserted in the field mid-way

between rows and central furrows to a depth of 8 cm (Hoben et al., 2011). At each sampling time, chamber height from soil surface was measured to account for headspace volume and then chambers were sealed with an air-tight, O-ring PVC lid, fitted with a Vacutainer serum vial septa (Becton-Dickinson, East Rutherford, NJ). Using a syringe, and mixing the headspace at each sampling, 10 mL gas samples were removed via the lid septa at time zero and at three additional 20-minute intervals, and stored in 5.9 mL gas Exetainers (Labco, Ceredigion United Kingdom) with headspace previously flushed. Samples were analyzed using gas chromatography (Hewlett Packard 5890 Series II, Rolling Meadows, IL, USA) with gases separated on a Poropak Q column (1.8 m, 80/100 mesh) at 80 °C. N_2O was analyzed with a ^{63}Ni electron capture detector at 350 °C.

Denitrification enzyme assay (DEA)

We measured the potential activity of denitrification enzymes (Groffman et al., 1999) with soil collected in November 2010. Five soil cores taken to 10 cm depth across each treatment were pooled and stored on ice for transport. In the laboratory soils were sieved to 4 mm, and 25 g were added each of two 120 ml flasks. To these, 25 mL of a solution with 1 mM KNO_3 and 2 mM succinate was added and flasks were stoppered. To one flask, the headspace was flushed completely with N_2, and to the other approximately 10% of head space gas was removed and replaced with acetylene. Flasks were incubated at 25 °C on a shaker at 200 rpm and 3 mL gas samples (into vials previously flushed with N_2) were taken at 15, 30, 45, and 60 min from each flask injected into a vial. Sample N_2O was measured on an HP 5890II gas chromatograph (Hewlett Packard, Rolling Meadows, Illinois) equipped with dual Poropak Q columns set to 80 °C and a ^{63}Ni electron capture detector (ECD). Incubations with acetylene give the rate of total denitrification (N_2O+N_2 production) and incubations without acetylene give

net N_2O production (N_2O production - reduction). The molar ratio of N_2O production ($N_2O/(N_2O+N_2)$) gives the efficiency of complete denitrification.

*Nitrite reductase (*nirK*) PCR and quality filtering*

A subsample of sieved soil collected above (for DEA) was frozen at -80°C and later DNA was extracted and purified using a MO BIO PowerSoil® DNA Isolation kit (MO BIO, Carlsbad, CA). The target nirK gene was selected following (Henry et al., 2004) and amplified with PCR using primers nirK-q1F (5'-RTY GGC GGH CAY GGC GA-3') and barcoded nirK-q1R (5'-GCC TCG ATC AGR TTG TGG TT -3') primers were modified as previously described (Beszteri et al., 2010; Sogin et al., 2006). Each PCR reaction occurred in of 25 µL volumes containing: 10 ng of purified DNA, 0.25 µM of each primer, 0.1 mM of each dNTP (Invitrogen, Carlsbad, CA), 2.5 mM of $MgCl_2$ (Invitrogen, Carlsbad, CA), and 2 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA), in 1x PCR Buffer (Invitrogen, Carlsbad, CA). Amplification conditions for the PCR were as follows: 95ºC for 5 min, followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 7 min. PCR amplification was performed using a PTC-200 Peltier Thermal Cycler (MJ Research, Waltham, MA). PCR products were gel purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI) and sequenced on a Roche 454 Junior Titanium according to the manufactures instructions. (454 Sequencing 2010 Manual (May)).

The *nirK* sequences were filtered in mothur v. 1.25.0 (Schloss et al., 2009) to remove barcode sequences, screen out low quality sequences (qaverage=25) and trim primers from the sequences. For this *nirK* region, aligned sequences have no gaps, so sequences not containing the expected 126 base pairs were removed as likely due to sequencing error, followed by clustering. Further community-level analysis of *nirK* sequence was performed in R (R Core

Team, 2016) using the VEGAN package (Oksanen et al., 2013). MEGA (Tamura et al., 2013) was used to assess *nirK* sequence clustering against reference sequences of ammonia oxidizing bacteria (AOB).

Statistical approach

Data for all soil analyses were examined for normality and heterogeneity of variance assumptions following Zuur et al. (2010). Data deviating from normality were log-transformed prior to analysis, and homogeneity of variance checked with Levine's test. Analysis of variance was calculated in R (The R Foundation for Statistical Consulting, Vienna, Austria) using the *lme4* package for linear, mixed-effect models (Bates et al., 2016), with time point nested within block as a random effect and crop rotation as a fixed effect. On ten gas sampling dates, measures were also taken for soil enzyme, mineral N, PMN and PMC. Along with soil moisture and presence of legume cover crop these variables were individually related to N_2O flux and then used to generate mixed model comparisons using an iterative approach and assessing goodness of fit with the Akaike Information Criterion (AIC). All results are reported as statistically significant at $\alpha = 0.05$, unless otherwise discussed.

RESULTS

Soil C and N

Among all treatments, mineral N was highest in CSW1cov, with a mean NH_4^+ of 2.5 µg NH_4^+-N g^{-1}, which was significantly higher than all other treatments ($P<0.05$), as was NO_3^- with a mean of 3.4 µg NO_3^--N g^{-1} (Table 4.2). Treatment cS had the second highest N values, with 2.2 µg NH_4^+-N g^{-1} (equal to or higher than all other treatments) and 2.7 µg NO_3^--N g^{-1} which was

nearly twice the NO_3^- of other non-covers. The two cover treatment, CSW2cov, also had higher NO_3^- than non-covers ($P<0.05$) at 2.2 μg NO_3^--N g^{-1}. The Fspring treatment had similar or lower NH_4^+ and NO_3^- concentrations than the non-covers (except for cS). By contrast, the Fspring plots and the cover cropped rotations had mean PMN between 8.6 and 9.0 μg NH_4^+-N g^{-1}, which was approximately twice the concentration of all other (non-cover) treatments ($P<0.01$). A similar contrast occurred in soil enzyme activities for NAG and TAP, which had significantly higher potentials in cover and fallow treatments (Table 4.2). Underlying these N values, the total SON was significantly higher in CSW1cov, 0.91 g N kg $soil^{-1}$, and CSW2cov, 0.93 g N kg $soil^{-1}$, than all other treatments with cC having the lowest SON (0.73 g N kg $soil^{-1}$), (Supplemental Table 4.1). Labile C as POXC was also significantly higher in CSW1cov 355 mg POX-C Kg^{-1} ($P<0.05$), followed by CSW2cov and Fspring. Similar to PMN, PMC was significantly higher in both cover treatments and Fspring ($P<0.01$) when compared to all other non-cover rotations (Table 4.2).

Nitrous oxide

In 2010, mean N_2O production in the rotations with cover crops was 3-4 times greater in magnitude than all other treatments (Table 4.3) with CSW2cov having the highest mean flux at 13.2 g N_2O-N ha^{-1} day^{-1}. Time points with the highest fluxes disproportionately influenced this difference between treatments though median flux rates across cropped rotations still ranged from 7.2 N_2O-N ha^{-1} day^{-1} in CSW2cov to 2.3 N_2O-N ha^{-1} day^{-1} in cC. Fspring had the lowest median flux at 1.2 N_2O-N ha^{-1} day^{-1} (Table 4.3). At least one of the cover cropped treatments was higher in N_2O flux at each measured time point except in November (Supplemental Table 4.5), with the strongest differences between treatments in the early part of the growing season

(Figure 4.1). Across all time points the Fspring treatment had N_2O fluxes at or below the magnitude of cropped treatments.

A similar pattern occurred in 2011, in which N_2O fluxes from cover cropped treatments diverged from all other rotations, especially with fluxes of larger magnitude in early spring (Figure 4.1). Overall, the mean N_2O flux was about a third as high in the second year as it was in 2010 across all treatments, with cover cropped treatments significantly higher than all other treatments ($P<0.05$) and with highest mean flux in CSW1cov with 5.2 N_2O-N ha^{-1} day^{-1} (Table 4.3). Similar flux patterns occurred among treatments as in the previous year, with cover cropped rotations having 2-3 times greater magnitudes of flux as treatments without cover (Table 4.3). Fluxes were again higher under cover crops at every time point, with median values in these rotations about double those of rotations without cover crops, and with the most significant differences across all rotations occurring in the spring and early summer measurements (Supplemental Table 4.5).

DEA

The denitrification potential and the relative rate of N_2O production differed across treatments based on results from DEA (Figure 4.2). In the anaerobic assay, N_2O production had high sample-to-sample variability and was not significantly different across treatments ($P=0.1033$), although cover cropped treatments tended to have higher mean fluxes, from 197 - 200 ng N_2O g^{-1} hr^{-1}, compared to cropped treatments without cover, from 122 - 159 ng N_2O g^{-1} hr^{-1}. However, when N_2 production was inhibited a clear and significant pattern emerged ($P=0.0042$) with cover crop treatments denitrifying about twice as much as treatments without cover, with CSW1cov and CSW2cov having the highest denitrification ($N_2O + N_2$) rates: 368

and 409 ng N_2O g^{-1} hr^{-1} respectively. This meant that the relative rate of N_2O production, [ΔN2O/Δ (N2O + N2)], or rN_2O was significantly lower in treatments with cover crops (P=0.0135), with a ratio between 0.51 - 0.59 compared to treatments without a history of cover which fell between 0.70 - 0.83 (Supplemental Table 4.2).

nirK *PCR amplicons*

Amplified *nirK* sequences differed significantly by rotation (Bray-Curtis, perMANOVA constrained by block, $p < 0.001$), with *nirK* cover cropped rotations distinct from no-cover rotations and from Fspring (Fig. 4.3). Clustering revealed two primary groups, one of which contained sequences identified as AOBs. Although these were a small portion of the amplicons (between 2 - 8 %), the percentage of sequences clustering with this group also differed by treatment (P=0.012), with treatments with cover crops having a higher percentage than no-cover treatments (Fig. 4.4).

DISCUSSION

Clear differences in N_2O flux emerged across the cropping systems gradient over the course of two years and appear to have been driven by changes in nutrient cycling and the soil microbial community, which were induced largely by the presence of cover crops. I hypothesized that treatments with a greater abundance of legumes in rotations would result in higher soil NO_3^- and lead to increased N_2O flux. While NO_3^- was significantly higher in rotations with cover crops, available NO_3^- did not always lead to higher N_2O. The presence of a legume cover crop in rotation, however, was the best predictor of higher gas flux in part due to higher potential mineralization of C and N. My hypothesis that cover crop rotations would shift

the phylogeny of *nirK* genes was supported and also suggested a change in pathway towards nitrifier production of N_2O under cover crops. Finally, I predicted that rotations with cover crops would increase the efficiency of heterotrophic denitrification and DEA indicated that even though *in situ* N_2O flux under these rotations was higher, rotations without cover crops were significantly less efficient at fully denitrifying. Along with influences from agronomic management, higher N_2O flux under rotations with cover crops appears to result from increased nutrient cycling and altered microbial pathways as a result of including overwintering legumes in rotation.

The presence of legumes in rotation increased mineral N, however while concentrations of soil NH_4^+ and NO_3^- were as high or higher in cS compared to rotations with cover crops (Table 4.1), cS had significantly lower N_2O flux, suggesting that substrate availability alone did not lead to the observed pattern in emissions. However, as contrasted to cS, the cover cropped rotations had higher nutrient cycling potential with twice the value of PMN, PMC and enzyme potentials associated with N mineralization (NAG+TAP), (Table 4.2), suggesting that decomposition and release of NH_4^+ may be more indicative of available substrate than ephemeral mineral N pools. However the fallow, Fspring, treatment also had PMN, PMC and enzyme potentials equivalent to cover cropped treatments and consistently had the lowest N_2O flux (Table 4.3, Supplemental Table 4.4). This contrast in available N pools and N cycling potential may be driven by the quality of plant detritus and degree of NH_4^+ mineralization versus immobilization by microorganisms during decomposition (Robertson and Groffman, 2015). Fspring had high mineralization potentials, but this treatment also had the highest soil C:N, 11.9, while cS had the lowest at 9.9 (Supplemental Table 4.1). In addition, while all the treatments were plowed in May of each year, the soil in cropped treatments continue to receive tillage

during the growing season for weed control, whereas Fspring was not be exposed to these potential mineralization events (Grandy and Robertson, 2007) and instead successional plant communities could scavenge available N. Thus, potentially greater soil N cycling, due in part to more intense management, may more adequately indicate substrate availability in cover cropped rotations that would lead to higher N_2O flux.

Legume cover crops increase total soil N (Syswerda et al., 2011) and both cover cropped rotations had significantly higher SON than all other treatments including Fspring (Supplemental Table 4.1). While the soil C:N in cover cropped rotations was not different from rotations without cover crops, spring plowing and incorporation of overwintering legumes introduces a high quality plant tissue which can rapidly release N into soil (Parton et al., 2007). Among individual variables, including available NO_3^-, the presence of legumes in rotation was the strongest predictor of N_2O flux ($P=0.014$), and when combined in a multivariate model, soil moisture and PMN best fit flux patterns (Table 4.4). In spite of this finding, the highly stochastic nature of soil N_2O emissions (Nol et al., 2010) means that individual flux events may have distinct underlying causes. In both years, peak flux events occurred in July (Figure 4.1). While relatively higher soil moisture prevailed during these periods (favoring heterotrophic denitrification), this is also a period of more intense management and tillage, approximately 6-8 weeks after cover crop incorporation. In this period the disproportionality higher emissions from rotations with cover crops may be exacerbated by higher mineralization from cover crops (Berthrong et al., 2013) stimulating both nitrification and denitrification. This confluence of factors can lead to intense N_2O flux events, or 'hot moments', (Molodovskaya et al., 2012), which can dominate temporal analysis and present a challenge to modeling denitrification (Groffman et al., 2009).

While short term environmental and management factors have an acute effect on soil trace gas emissions (Gelfand et al., 2016), they essentially act on the activity of the underlying microbial community (Cavigelli and Robertson, 2000). My data suggests that microbial communities responsible for N_2O flux are significantly different in rotations with cover crops. Under anoxic conditions favoring heterotrophic denitrification, the relative rate of N_2O to N_2 production, rN_2O, was between 16 - 39% higher in rotations without cover crops, suggesting that in the field, incomplete denitrification could lead to high relative rates of N_2O. This suggests that denitrifiers in cover crop rotations are better adapted to coupling C respiration to complete denitrification (as DEA is saturated with respect to labile C substrate). This may be a result of greater availability of C, as POXC was higher in these rotations (Table 4.2), or better coupling of available C with NO_3^- as PMC was significantly higher under cover crops and could supply more substrate during ideal conditions for denitrification.

Functional differences in the denitrifier community in cover crop rotations was further supported by the significant difference in the structure of *nirK* genes (Figure 4.3). While for all soils the majority of these amplicons clustered with *nirK* from denitrifying bacteria, a portion clustered with AOB lineages, and rotations with cover crops had between 2-10 times greater relative abundance of AOB *nirK* (Figure 4.4). The diversity of genes underlying denitrification pathways is broad and only partially described (Jones et al., 2008), such that the *nirK* genes captured in this study represent only a fraction of the phylogenetic distribution of nitrite reductases. However, along with a general shift in the denitrifier community, the increase in *nirK* from AOB lineages suggests that nitrification may be an important source of N_2O flux in rotations with cover crops. Indeed, even in grassland systems fertilization can increase the abundance of AOB leading to higher N_2O from nitrification (Avrahami and Bohannan, 2009).

Increasingly, nitrification has been identified as a potent source of N_2O in agricultural systems (Zhu et al., 2013).

The structure of denitrifier communities broadly conforms to soil and land management (Enwall et al., 2010), and their diversity influences ecosystem function (Hallin et al., 2009), such that *nir*-type genes may be used in predictive models of denitrification (Powell et al., 2015). While individual plant species can directly influence the structure of *nirK* genes and alter N_2O production (Abalos et al., 2014; Bremer et al., 2009), my study suggests that even in soil under the same grain crop rotation, and without external inputs of mineral N, that cover crops alter the structure and function of the denitrifier community.

A unique advantage to this study was the absence of external inputs or other differences in management practices, such that the primary variable differing across all treatments was the legacy of each specific crop rotation. The increase in N_2O flux due to cover crops may be both direct and indirect. Previously on this site, Tiemann et al. (2015) found that rotations with cover crops had higher soil aggregation in spite of identical tillage regimes. Greater soil aggregation alters microbial community structure as well as the oxygen environment in and outside of soil aggregates (Paul et al., 2015), and has been found to influence soil N_2O flux (Miller et al., 2009). Soil structure may also interact with high substrate availability and soil moisture especially when conditions align for high flux events.

Cover crops can improve field-scale N mass balances, particularly through reduced NO_3^- leaching (Tonitto et al., 2006), and can immobilize NO_3^- that might otherwise available for denitrification (McSwiney et al., 2010). A recent long term study of a corn-soybean rotation with and without overwintering rye cover crop showed no difference in overall N_2O flux, but significant effects of reduced N_2O in rye treatments when comparing paired flux events in

specific annual periods, especially in soybean (which was not fertilized as was the corn) (Parkin et al., 2016). Apart from just N availability influenced directly by the rye, temporal changes in N_2O flux (Verma et al., 2006) may also be related to gene expression of denitrifiers in response to seasonality (Németh et al., 2014) and management events (Wessen et al., 2011). In the current study, the inclusion of rye cover in the most complex, CSW2cov, treatment did reduce NH_4^+ and NO_3^- compared to CSW1cov with only a legume cover, even while PMN was the same (Table 4.2). However there was no difference in mean N_2O flux (Table 4.3), and no clear trend in paired flux events between rotations with cover, especially when considering peak 'hot moment' events. Still, with few time points in this study, especially in key seasonal periods, the effect of reduced NO_3^- availability in treatments with rye may have some mitigating effect on N_2O emissions. Higher available N, likely through increased mineralization, along with incorporation of legume residues may have an overriding influence on N_2O (Mitchell et al., 2013) similar to other low input row crop experiments - with cover cropped systems that showed increased N_2O emissions even outside of tillage or litter incorporation events (Peyrard et al., 2016).

While the scope of N_2O measurements in this study did reveal consistent patterns across treatments, and the potential for extremely high flux events under cover crops, the magnitude of N_2O rates were similar to those measured in conventionally managed and low-input row crop systems at this experimental site (Gelfand et al., 2016; Oates et al., 2016). Interestingly adjacent, conventionally managed row crop treatments at this site appeared to have the highest proportion of AOB *nirK* when compared to the gradient in the current study (Supplemental 4.3). This may indicate that differences in N use between these types of cropping systems may control potential sources of N_2O. While the presence of cover crops in this study led to more complete denitrification than rotations without cover, knowledge of the microbial pathways leading to N_2O

flux in rotations with cover crops would directly impact further strategies to mitigate N_2O emissions. Among numerous management approaches, greater mineralization and nitrification might dictate greater emphasis on synchronizing N release and plant uptake (Grandy et al., 2012) while increased denitrification might require careful timing of N immobilization (McSwiney et al., 2010). Increasing N use efficiency in agroecosystems remains an overarching challenge to reduce leaching and N_2O emissions (Robertson and Vitousek, 2009; Snyder et al., 2014). This study indicates that while cover crops increase efficiency in denitrification, they also pose a unique set of challenges to reduce N_2O emissions in low-input agroecosystems.

ACKNOWLEDGEMENTS

Support for this research was also provided by U.S. Department of Agriculture Soil Processes Program, grant # 2009-65107-05961, and the National Science Foundation Long-term Ecological Research Program (DEB 1027253) at the Kellogg Biological Station.

APPENDIX

Table 4.1 Crop rotations sampled on the KBS Biodiversity Gradient Experiment

ID	Treatment	Rotation	Species / yr.	Species / rot.
1	cC	corn	1	1
2	cS	soy	1	1
3	CS	corn + soy	1	2
4	CSW	corn + soy + wheat	1-2	3
5	CSW1cov	corn + soy + wheat + clover	2-3	4
6	CSW2cov	corn + soy + wheat + clover + rye	3-4	5

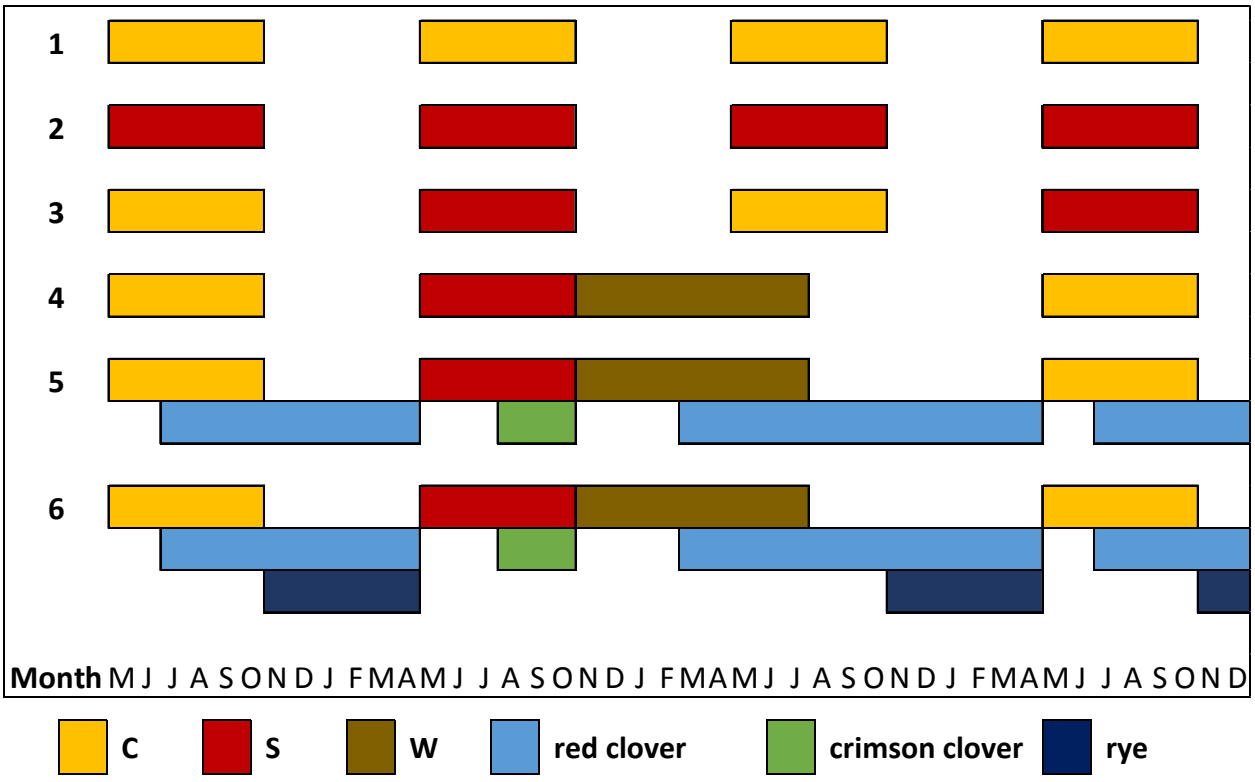


Table 4.2 Potentially mineralizable N (PMN), N-acetyl-glucosaminidase + tyrosine amino peptidase (NAG+TAP), inorganic N (NH_4^+-N and NO_3^--N), potentially mineralizable C (PMC) and permanganate oxidizable (POX) C on the biodiversity gradient treatments (see table 4.1 for treatment acronyms) for the two study years. Values are means (standard error), n=4 replicate blocks. Significance of treatment effect: * $P \leq 0.05$, ** $P = 0.001$ to 0.01.

Rotation	PMN	NAG+TAP	NH_4^+-N	NO_3^--N	PMC	POXC
	μg NH_4^+-N g^{-1}	nmol g^{-1} h^{-1}	μg NH_4^+-N g^{-1}	μg NO_3^--N g^{-1}	μg CO_2-C g^{-1}	mg POX-C Kg^{-1}
cS	4.7(0.4)	31.8(2.0)	2.2(0.3)	2.7(0.2)*	32.0(2.5)	276(20)
cC	3.8(0.3)	32.4(2.2)	1.6(0.2)	1.5(0.1)	29.4(2.3)	263(16)
CS	4.7(0.4)	31.0(2.0)	1.5(0.2)	1.7(0.2)	35.3(3.1)	277(16)
CSW	5.1(0.4)	34.6(2.6)	2.2(0.3)	1.8(0.2)	34.7(3.2)	278(14)
CSW1cov	8.6(0.5)**	45.7(3.2)*	2.5(0.3)*	3.4(0.4)**	51.5(4.0)**	355(15)*
CSW2cov	9.0(0.6)**	51.7(3.7)**	1.7(0.1)	2.2(0.2)*	49.3(3.9)**	323(13)
Fspring	8.6(0.6)**	46.8(4.0)*	1.9(0.2)	1.1(0.2)	50.4(4.6)**	301(19)

Table 4.3 *In situ* nitrous oxide fluxes in the biodiversity gradient treatments for the two study years. Median, maximum (Max) and mean (standard error) for n=4 replicate blocks, followed by letters denoting significant differences between treatments. See table 4.1 for treatment acronyms.

Year	Rotation	Median	Max	Mean (se)
			g N_2O-N ha^{-1} day^{-1}	
2010	cS	4.5	10.7	4.6 $(0.8)_{ab}$
	cC	2.3	11.2	4.2 $(0.8)_{ab}$
	CS	3.1	11.8	3.5 $(0.6)_{a}$
	CSW	4.3	26.5	4.4 $(0.9)_{ab}$
	CSW1cov	3.6	41.1	10.5 $(2.5)_{bc}$
	CSW2cov	7.2	58.6	13.2 $(3.4)_{c}$
	Fspring	1.2	9.5	2.5 $(0.6)_{a}$
2011	cS	1.5	3.0	1.3 $(0.2)_{a}$
	cC	1.0	10.5	2.1 $(0.7)_{a}$
	CS	1.1	7.0	1.5 $(0.3)_{a}$
	CSW	0.8	7.7	1.6 $(0.4)_{a}$
	CSW1cov	2.3	34.0	5.2 $(1.7)_{b}$
	CSW2cov	2.6	29.4	4.6 $(1.2)_{b}$
	Fspring	1.7	4.1	2.0 $(0.8)_{a}$

Table 4.4 Model reduction to best fit with N_2O as response variable and predictor variables presence of cover (0,1,2), moisture content, nitrate (NO_3^-), ammonium (NH_4^+), potentially mineralizable N (PMN) and N-acetyl-glucosaminidase + tyrosine amino peptidase (NAG+TAP). Random variables in the equation are treatment block and time point of flux measurement (block:time point).

Model	AIC
N_2O ~ cover + moisture + NO_3^- + NH_4^+ + PMN + NAG+TAP + (1 | block:time point)	269.17
N_2O ~ cover + moisture + NO_3^- + PMN + NAG+TAP + (1 | block:time point)	262.68
N_2O ~ cover + moisture + NO_3^- + PMN + (1 | block:time point)	249.51
N_2O ~ cover + moisture + PMN + (1 | block:time point)	244.18

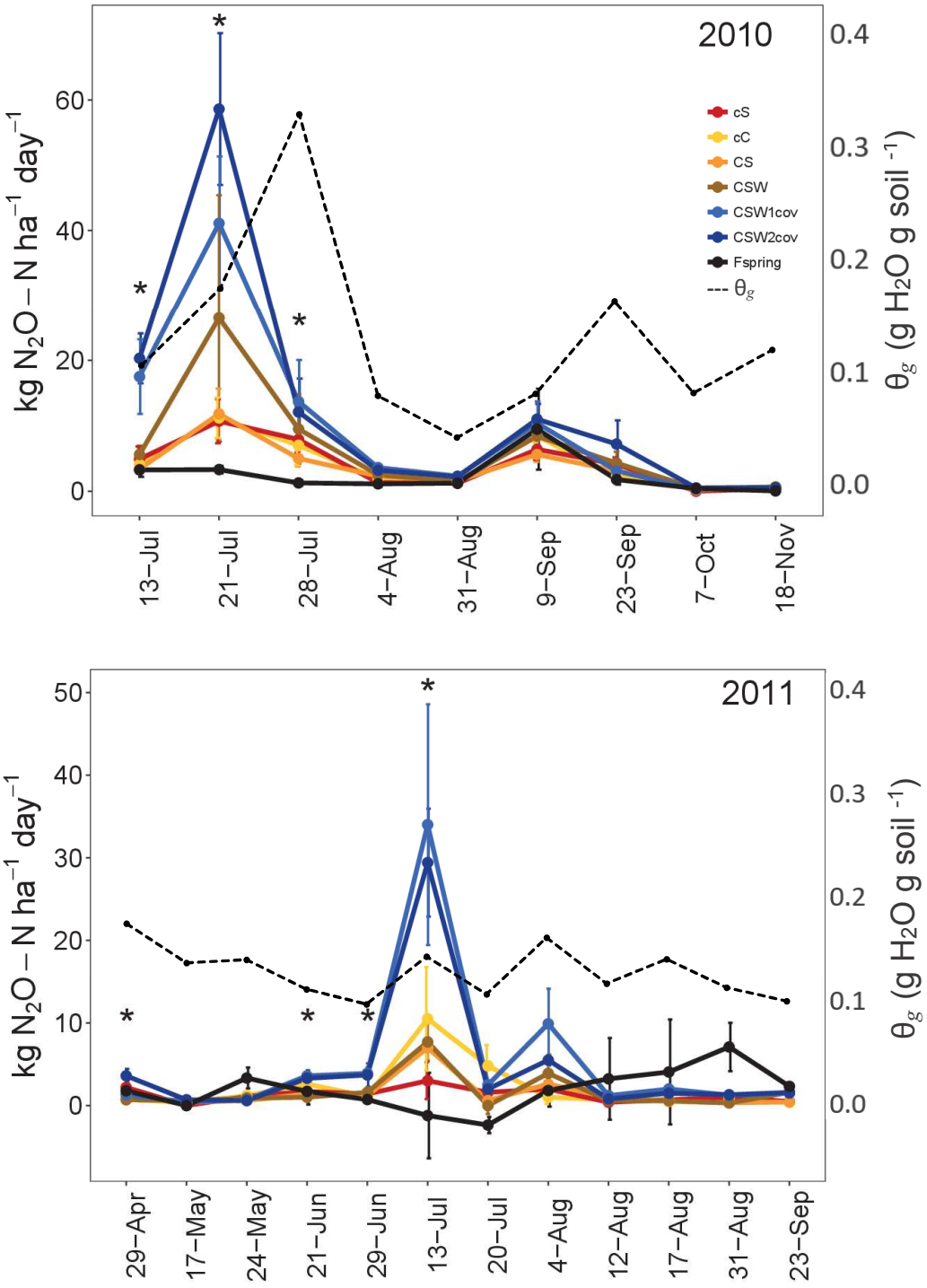


Figure 4.1 *In situ* N_2O flux and gravimetric moisture (θ_g) for KBS biodiversity gradient for two years. Time points are means with standard error for N_2O, n=4, and means across all treatments for moisture. Asterisks (*) show significant differences ($P \leq 0.05$) by treatments at a specific time point. See table 4.1 for treatment acronyms.

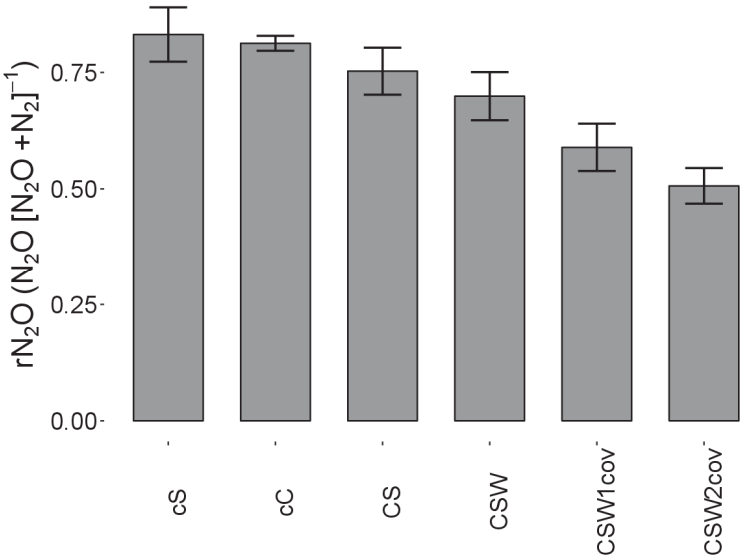


Figure 4.2 Denitrification enzyme assay (DEA) on the KBS biodiversity gradient. Bars represent the mean rN_2O or the relative rate of nitrous oxide flux per total denitrification (including estimated N_2) with standard error, n=4. See table 4.1 for treatment acronyms.

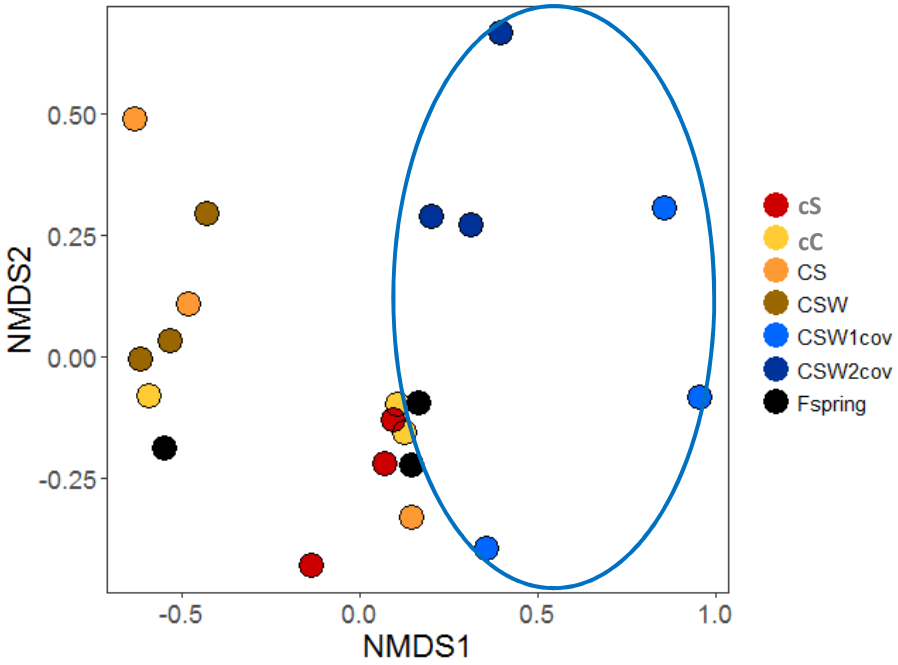


Figure 4.3 Non-metric multidimensional scaling (NMDS) plot of nitrite reductase (nirK) gene amplicons from crop rotations, (n=3) on the biodiversity gradient experiment (see table 4.1 for treatment acronym). Blue ellipse encircles treatments with cover crops. Amplicon count data were transformed using Bray-Curtis dissimilarity matrix. Stress was 0.12349.

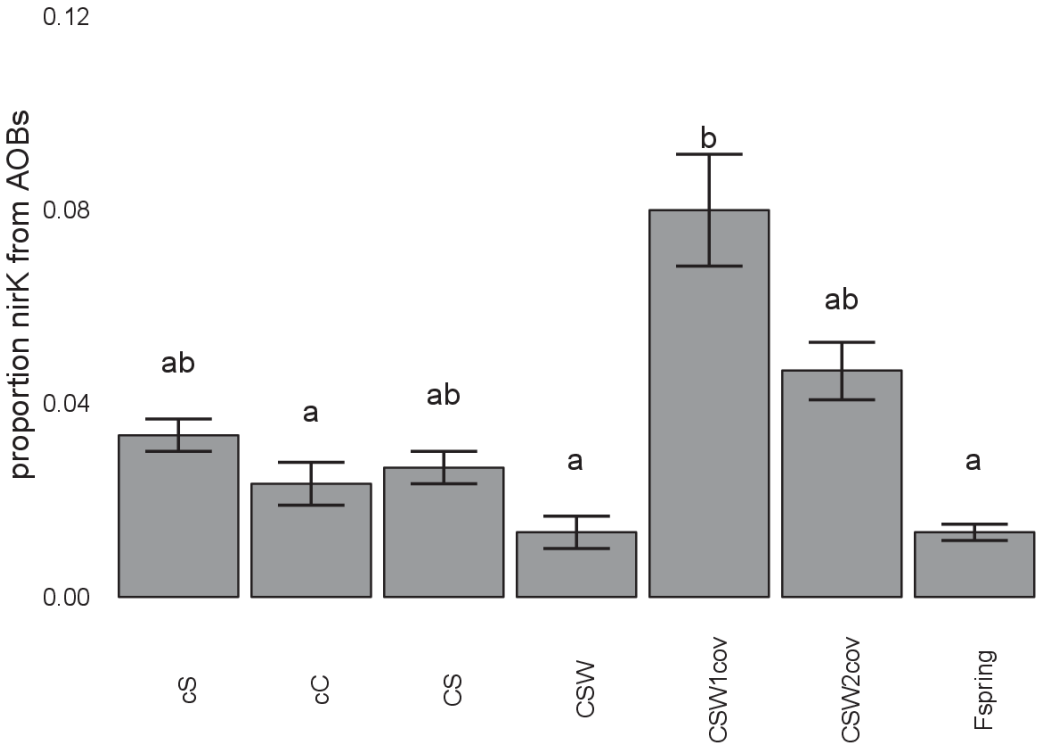


Figure 4.4 Proportion of nitrite reductase (nirK) gene amplicons from AOBs for crop rotations on the biodiversity gradient experiment (n=3). See table 4.1 for treatment acronyms.

Supplemental Table 4.1 Soil organic C (SOC) and N (SON), and their ratio (C:N), in the biodiversity gradient treatments. Values are means (standard error), n=4 replicate blocks. Significance of treatment effect: * $P \leq 0.05$. See table 4.1 for treatment acronym.

Rotation	SOC	SON	C:N
	g C kg soil^{-1}	g N kg soil^{-1}	
cS	7.93(0.96)	0.80(0.09)	9.9(0.13)
cC	7.56(0.61)	0.73(0.06)	10.5(0.46)
CS	7.95(0.96)	0.76(0.12)	10.8(0.58)
CSW	7.93(0.60)	0.77(0.07)	10.5(0.54)
CSW1cov	9.30(0.35)*	0.91(0.05)*	10.3(0.45)
CSW2cov	9.38(0.38)*	0.93(0.06)*	10.2(0.38)
Fspring	10.42(1.35)	0.86(0.07)	11.9(0.81)

Supplemental Table 4.2 Denitrification enzyme potential assay (DEA) showing N_2O flux, total denitrification ($N_2O + N_2$) and relative rates of N_2O production (rN_2O) on rotations in the biodiversity gradient experiment. Values are means (standard error), n=4 replicate blocks, with overall treatment significance above values and letters denoting significant difference between rotational treatments at $P \leq 0.05$. See table 4.1 for treatment acronym.

Rotation	N_2O ng N_2O g^{-1}-hr^{-1} (P=0.1033)	$N_2O + N_2$ ng N_2O g^{-1}-hr^{-1} (P=0.0042)	rN_2O $N_2O/(N_2O + N_2)$ (P=0.0135)
cS	159 (19)	193 $(8)_{ab}$	0.83 $(0.1)_b$
cC	122 (28)	151 $(34)_a$	0.81 $(0.0)_b$
CS	117 (9)	164 $(25)_a$	0.75 $(0.1)_{ab}$
CSW	130 (23)	191 $(32)_{ab}$	0.70 $(0.1)_{ab}$
CSW1cov	197 (39)	368 $(110)_{bc}$	0.59 $(0.1)_{ab}$
CSW2cov	200 (16)	409 $(30)_c$	0.51 $(0.1)_a$
Fspring	177 (25)	167 $(67)_{ab}$	1.82 (0.9) ¥

¥ Values for Fpring were left out of rN_2O test for significance and treatment comparisons.

Supplemental Table 4.3 Total number of *nirK* sequence reads, and within that pool the number of sequences that clustered with known sequences of *nirK* from ammonia oxidizing bacteria (AOBs). Treatments are from the biodiversity cropping experiment followed by a simultaneous comparison with sequence from a nearby deciduous forest (DF) and conventional row crop system (T1), for the years 2008 and 2009. See table 4.1 for treatment acronym.

Treatment	Field replicate treatment #	Total # of *nirK* sequence reads	# of *nirK* sequence clustering with AOB
cS	1	440	10
cS	2	2740	103
cS	3	3938	152
cC	1	1527	36
cC	2	1682	67
cC	3	1713	10
CS	1	1244	22
CS	2	1362	51
CS	3	228	4
CSW	1	258	1
CSW	2	1647	36
CSW	3	241	4
CSW1cov	1	623	49
CSW1cov	2	919	112
CSW1cov	3	2566	105
CSW2cov	1	1548	39
CSW2cov	2	611	41
CSW2cov	3	513	19
Fspring	1	2033	18
Fspring	2	1804	19
Fspring	3	1856	38
DF - 2008	1	2991	28
DF - 2008	2	1817	2
DF - 2009	1	1934	7
DF - 2009	2	1038	0
T1 - 2008	1	2121	431
T1 - 2008	2	883	235
T1 - 2009	1	786	67
T1 - 2009	2	2440	286

Supplemental Table 4.4 *In situ* nitrous oxide fluxes in the biodiversity gradient treatments for individual time points over two years with mean (standard error) for n=4 replicate blocks, followed by letters denoting significant differences between treatments. See table 4.1 for treatment acronym.

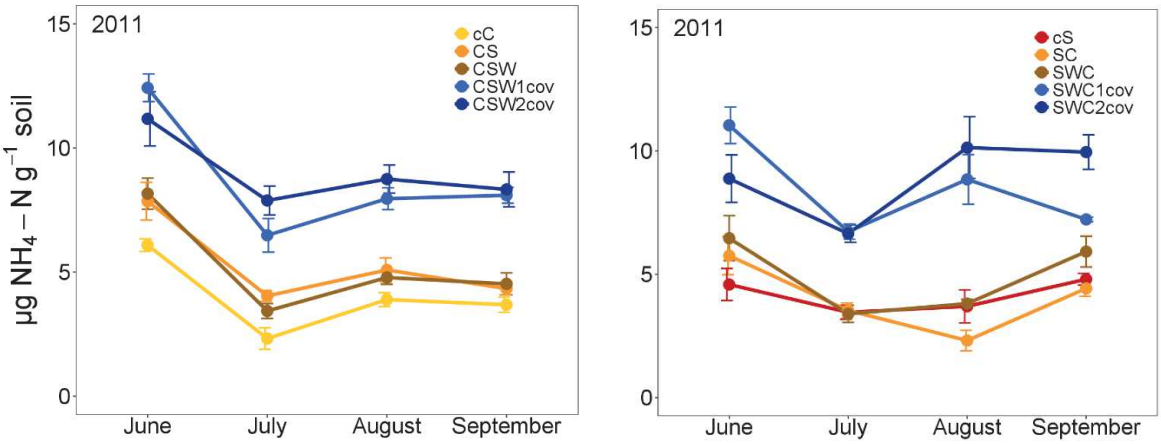
2010	g N_2O-N ha^{-1} day^{-1}								
Rotation	07/13/10	07/21/10	07/28/10	08/04/10	08/31/10	09/09/10	09/23/10	10/07/10	11/18/10
cS	4.9 (1.9)$_a$	10.7 (3.3)$_a$	7.9 (2.0)	1.3 (0.3)$_a$	1.2 (0.5)	6.4 (1.6)	4.1 (1.1)	n.a.	0.4 (0.3)
cC	4.1 (0.5)$_a$	11.2 (3.1)$_{ab}$	7.0 (3.1)	1.9 (0.5)$_{ab}$	1.5 (0.4)	8.4 (1.0)	2.3 (0.3)	0.3 (0.1)	0.7 (0.3)
CS	3.4 (0.5)$_a$	11.8 (3.9)$_{ab}$	5.0 (1.2)	2.3 (0.6)$_{ab}$	1.6 (0.3)	5.6 (1.1)	3.1 (0.6)	0.2 (0.1)	0.5 (0.4)
CSW	5.6 (1.3)$_a$	26.5 (18.9)$_{ab}$	9.5 (5.3)	2.5 (0.8)$_{ab}$	1.5 (0.2)	8.4 (1.5)	4.3 (1.7)	0.4 (0.1)	0.3 (0.3)
CSW1cov	17.5 (5.7)$_b$	41.1 (10.3)$_{ab}$	13.7 (6.3)	3.6 (0.4)$_b$	2.3 (0.4)	10.4 (3.3)	3.1 (0.8)	0.3 (0.1)	0.4 (0.2)
CSW2cov	20.3 (3.8)$_b$	58.6 (11.6)$_b$	12.1 (5.1)	3.2 (0.3)$_{ab}$	2.2 (0.2)	11.0 (2.3)	7.2 (3.6)	0.5 (0.1)	0.6 (0.2)
Fspring	3.2 (1.0)	3.3 (0.6)	1.3 (0.4)	1.1 (0.4)	1.2 (0.0)	9.5 (6.2)	1.8 (0.8)	0.4 (0.0)	0.0 (0.3)

Supplemental Table 4.4 (cont'd)

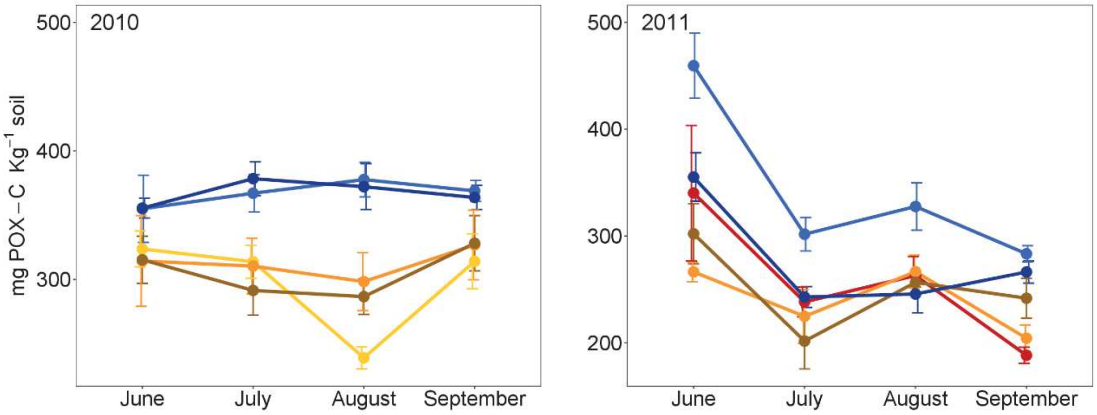
2011	g N_2O-N ha^{-1} day^{-1}					
Rotation	04/29/11	05/17/11	05/24/11	06/21/11	06/29/11	07/13/11
cS	2.2 $(0.3)_{ab}$	0.0 (0.2)	1.1 (0.4)	1.7 $(0.2)_{ab}$	1.4 $(0.2)_{ab}$	3.0 $(2.2)_{a}$
cC	0.8 $(0.2)_{b}$	0.3 (0.2)	1.2 (0.4)	2.6 $(0.7)_{ab}$	1.1 $(0.4)_{a}$	10.5 $(6.3)_{ab}$
CS	1.4 $(0.5)_{ab}$	0.4 (0.2)	0.6 (0.3)	1.4 $(0.3)_{ab}$	1.2 $(0.2)_{ab}$	7.0 $(1.3)_{ab}$
CSW	0.7 $(0.4)_{b}$	0.6 (0.2)	0.9 (0.3)	1.0 $(0.2)_{a}$	1.6 $(0.6)_{ab}$	7.7 $(2.3)_{ab}$
CSW1cov	1.3 $(0.5)_{b}$	0.4 (0.1)	0.7 (0.3)	3.6 $(0.4)_{b}$	3.9 $(0.7)_{b}$	34.0$(14.6)_{b}$
CSW2cov	3.6 $(0.8)_{a}$	0.7 (0.2)	0.6 (0.3)	3.3 $(1.0)_{ab}$	3.7 $(1.4)_{ab}$	29.4 $(6.5)_{b}$
Fspring	1.8 (1.3)	n.a	3.3 (1.3)	1.7 (1.6)	0.7 (0.2)	-1.2 (5.2)

Supplemental Table 4.4 (cont'd)

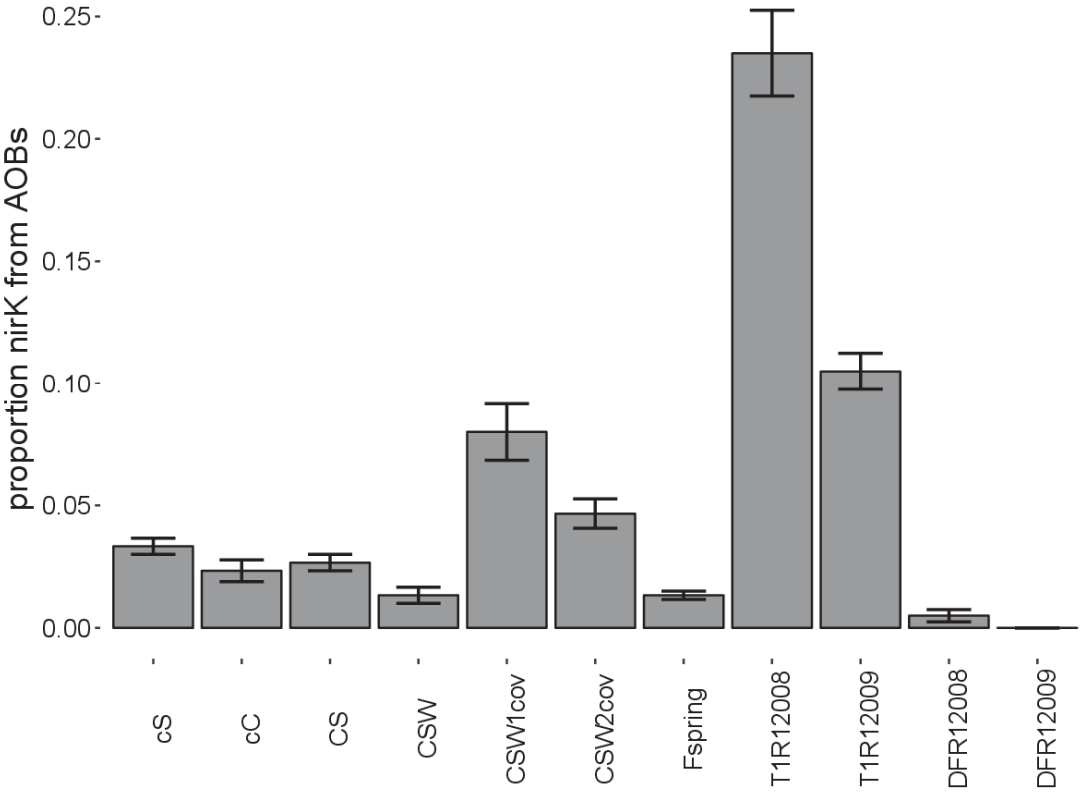
07/20/11	08/04/11	08/12/11	08/17/11	08/31/11	09/23/11
1.6 (0.9)	2.0 (0.5)	0.4 (0.3)	0.7 (0.3)	1.0 (0.1)	0.5 (0.1)
4.8 (2.5)	1.0 (0.8)	0.7 (0.3)	0.7 (0.2)	0.9 (0.1)	0.4 (0.3)
0.6 (1.2)	2.6 (0.9)	1.0 (0.3)	0.5 (0.2)	0.3 (0.2)	0.4 (0.3)
0.4 (1.0)	3.9 (2.2)	0.6 (0.3)	0.6 (0.2)	0.3 (0.0)	1.6 (0.2)
2.5 (0.5)	9.9 (4.2)	1.2 (0.4)	2.0 (0.5)	1.3 (0.1)	1.5 (0.2)
2.0 (0.9)	5.5 (0.7)	0.8 (0.2)	1.5 (0.6)	1.3 (0.2)	1.6 (0.2)
-2.4 (1.0)	1.8 (1.9)	3.2 (4.9)	4.1 (6.4)	7.1 (2.9)	2.3 (0.4)



Supplemental Figure 4.1 Potentially mineralizable N (PMN) on the biodiversity gradient treatments for the two study years. All points are means across blocks (n=4) with standard errors for a corn year (left) and following soybean year (right). See table 4.1 for treatment acronym.



Supplemental Figure 4.2 Permanganate oxidizable (POX) C on the biodiversity gradient treatments for the two study years. All points are means across blocks (n=4) with standard errors for a corn year (left) and following soybean year (right). See table 4.1 for treatment acronym.



Supplemental Figure 4.3 Proportion of nitrite reductase (*nirK*) gene amplicons from AOBs in crop rotations on the biodiversity gradient experiment (n=3), (see table 4.1 for treatment acronym) shown with treatments from the KBS Main Cropping Experiments (T1, conventional management for 2008 and 2009) and Deciduous Forest (DF).

REFERENCES

REFERENCES

Abalos, D., De Deyn, G.B., Kuyper, T.W., van Groenigen, J.W., 2014. Plant species identity surpasses species richness as a key driver of N2O emissions from grassland. Glob Change Biol 20, 265–275. doi:10.1111/gcb.12350

Avrahami, S., Bohannan, B.M., 2009. N2O emission rates in a California meadow soil are influenced by fertilizer level, soil moisture and the community structure of ammonia-oxidizing bacteria. Global Change Biology 15, 643–655. doi:10.1111/j.1365-2486.2008.01727.x

Berthrong, S.T., Buckley, D.H., Drinkwater, L.E., 2013. Agricultural Management and Labile Carbon Additions Affect Soil Microbial Community Structure and Interact with Carbon and Nitrogen Cycling. Microb Ecol 1–13. doi:10.1007/s00248-013-0225-0

Beszteri, B., Temperton, B., Frickenhaus, S., Giovannoni, S.J., 2010. Average genome size: a potential source of bias in comparative metagenomics. The ISME journal 4, 1075–1077.

Blesh, J., Drinkwater, L.E., 2013. The Impact of Nitrogen Source and Crop Rotation on Nitrogen Mass Balances in the Mississippi River Basin. Ecological Applications. doi:10.1890/12-0132.1

Bremer, C., Braker, G., Matthies, D., Beierkuhnlein, C., Conrad, R., 2009. Plant presence and species combination, but not diversity, influence denitrifier activity and the composition of nirK□type denitrifier communities in grassland soil. FEMS microbiology ecology 70, 377–387.

Casciotti, K.L., Ward, B.B., 2001. Dissimilatory Nitrite Reductase Genes from Autotrophic Ammonia-Oxidizing Bacteria. Applied and Environmental Microbiology 67, 2213–2221.

Cavigelli, M.A., Robertson, G.P., 2000. The Functional Significance of Denitrifier Community Composition in a Terrestrial Ecosystem. Ecology 81, 1402–1414. doi:10.1890/0012-9658(2000)081[1402:TFSODC]2.0.CO;2

Doane, T.A., Horwáth, W.R., 2003. Spectrophotometric Determination of Nitrate with a Single Reagent. Analytical Letters 36, 2713–2722. doi:10.1081/AL-120024647

Drinkwater, L., Snapp, S., 2007. Understanding and managing the rhizosphere in agroecosystems. Elsevier: Burlington, MA, USA.

Drinkwater, L.E., Cambardella, C.A., Reeder, J.D., Rice, C.W., 1996. Potentially mineralizable nitrogen as an indicator of biologically active soil nitrogen, in: Methods for Assessing Soil Quality. Soil Science Society of America, Madison, WI, pp. 217–229.

Dybzinski, R., Fargione, J.E., Zak, D.R., Fornara, D., Tilman, D., 2008. Soil fertility increases with plant species diversity in a long-term biodiversity experiment. Oecologia 158, 85–93. doi:10.1007/s00442-008-1123-x

Enwall, K., Throbäck, I.N., Stenberg, M., Söderström, M., Hallin, S., 2010. Soil Resources Influence Spatial Patterns of Denitrifying Communities at Scales Compatible with Land Management. Applied and Environmental Microbiology 76, 2243–2250.

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, 613–623.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R., Vöosmarty, C.J., 2004. Nitrogen Cycles: Past, Present, and Future. Biogeochemistry 70, 153-226–226.

Gelfand, I., Shcherbak, I., Millar, N., Kravchenko, A.N., Robertson, G.P., 2016. Long-term nitrous oxide fluxes in annual and perennial agricultural and unmanaged ecosystems in the upper Midwest USA. Glob Change Biol 22, 3594–3607. doi:10.1111/gcb.13426

Grandy, A.S., Robertson, G.P., 2007. Land-Use Intensity Effects on Soil Organic Carbon Accumulation Rates and Mechanisms. Ecosystems 10, 59–74. doi:10.1007/s10021-006-9010-y

Groffman, P.M., Altabet, M.A., Böhlke, J.K., Butterbach-Bahl, K., David, M.B., Firestone, M.K., Giblin, A.E., Kana, T.M., Nielsen, L.P., Voytek, M.A., 2006. Methods for measuring denitrification: diverse approaches to a difficult problem. Ecological Applications 16, 2091–2122.

Groffman, P.M., Butterbach-Bahl, K., Fulweiler, R.W., Gold, A.J., Morse, J.L., Stander, E.K., Tague, C., Tonitto, C., Vidon, P., 2009. Challenges to incorporating spatially and temporally explicit phenomena (hotspots and hot moments) in denitrification models. Biogeochemistry 93, 49–77. doi:10.1007/s10533-008-9277-5

Groffman, P.M., Holland, E.A., Myrold, D.., Robertson, G.P., Xiaoming, Z., 1999. Denitrification, in: Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York.

Hallin, S., Jones, C.M., Schloter, M., Philippot, L., 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. ISME J 3, 597–605.

Haney, R., Brinton, W., Evans, E., 2008. Soil CO2 respiration: Comparison of chemical titration, CO2 IRGA analysis and the Solvita gel system. Renewable Agriculture and Food Systems 23, 171.

Haney, R.L., Haney, E.B., 2010. Simple and rapid laboratory method for rewetting dry soil for incubations. Communications in soil science and plant analysis 41, 1493–1501.

Henry, S., Baudoin, E., Lopez-Gutierrez, J.C., Martin-Laurent, F., Brauman, A., Philippot, L., 2004. Quantification of denitrifying bacteria in soils by nirK gene targeted real-time PCR. Journal of Microbiological Methods 59, 327–335. doi:doi: DOI: 10.1016/j.mimet.2004.07.002

Hoben, J.P., Gehl, R.J., Millar, N., Grace, P.R., Robertson, G.P., 2011. Nonlinear nitrous oxide (N2O) response to nitrogen fertilizer in on-farm corn crops of the US Midwest. Global Change Biology 17, 1140–1152. doi:10.1111/j.1365-2486.2010.02349.x

IPCC, 2013. , The Physical Science Basis. Summary for Policymakers. United Nations.

Jones, C.M., Stres, B., Rosenquist, M., Hallin, S., 2008. Phylogenetic Analysis of Nitrite, Nitric Oxide, and Nitrous Oxide Respiratory Enzymes Reveal a Complex Evolutionary History for Denitrification. Mol Biol Evol 25, 1955–1966. doi:10.1093/molbev/msn146

Kool, D.M., Van Groenigen, J.W., Wrage, N., 2011. Chapter Six - Source Determination of Nitrous Oxide Based on Nitrogen and Oxygen Isotope Tracing: Dealing with Oxygen Exchange, in: Stein, M.G.K. and L.Y. (Ed.), Methods in Enzymology, Research on Nitrification and Related Processes, Part B. Academic Press, pp. 139–160. doi:10.1016/B978-0-12-386489-5.00006-3

Leininger, S., Urich, T., Schloter, M., Schwark, L., Qi, J., Nicol, G.W., Prosser, J.I., Schuster, S.C., Schleper, C., 2006. Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442, 806–809. doi:10.1038/nature04983

Liebman, Davis, 2000. Integration of soil, crop and weed management in low-external-input farming systems. Weed Research 40, 27–47. doi:10.1046/j.1365-3180.2000.00164.x

McSwiney, C.P., Snapp, S.S., Gentry, L.E., 2010. Use of N immobilization to tighten the N cycle in conventional agroecosystems. Ecological Applications 20, 648–662. doi:10.1890/09-0077.1

Miller, M.N., Zebarth, B.J., Dandie, C.E., Burton, D.L., Goyer, C., Trevors, J.T., 2009. Denitrifier Community Dynamics in Soil Aggregates under Permanent Grassland and Arable Cropping Systems. Soil Science Society of America Journal 73, 1843–1851. doi:10.2136/sssaj2008.0357

Molodovskaya, M., Singurindy, O., Richards, B.K., Warland, J., Johnson, M.S., Steenhuis, T.S., 2012. Temporal Variability of Nitrous Oxide from Fertilized Croplands: Hot Moment Analysis. Soil Science Society of America Journal 76, 1728–1740. doi:10.2136/sssaj2012.0039

Németh, D.D., Wagner-Riddle, C., Dunfield, K.E., 2014. Abundance and gene expression in nitrifier and denitrifier communities associated with a field scale spring thaw N2O flux event. Soil Biology and Biochemistry 73, 1–9. doi:10.1016/j.soilbio.2014.02.007

Nol, L., Heuvelink, G.B.M., Veldkamp, A., de Vries, W., Kros, J., 2010. Uncertainty propagation analysis of an N2O emission model at the plot and landscape scale. Geoderma 159, 9–23. doi:10.1016/j.geoderma.2010.06.009

Oates, L.G., Duncan, D.S., Gelfand, I., Millar, N., Robertson, G.P., Jackson, R.D., 2016. Nitrous oxide emissions during establishment of eight alternative cellulosic bioenergy cropping systems in the North Central United States. GCB Bioenergy 8, 539–549. doi:10.1111/gcbb.12268

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., others, 2013. Vegan: Community Ecology Package.

Parkin, T.B., Kaspar, T.C., Jaynes, D.B., Moorman, T.B., 2016. Rye Cover Crop Effects on Direct and Indirect Nitrous Oxide Emissions. Soil Science Society of America Journal 80, 1551–1559. doi:10.2136/sssaj2016.04.0120

Parton, W., Silver, W.L., Burke, I.C., Grassens, L., Harmon, M.E., Currie, W.S., King, J.Y., Adair, E.C., Brandt, L.A., Hart, S.C., Fasth, B., 2007. Global-Scale Similarities in Nitrogen Release Patterns During Long-Term Decomposition. Science 315, 361–364. doi:10.1126/science.1134853

Paul, E.A., Kravchenko, A., Grandy, A.S., Morris, S., 2015. Soil organic matter dynamics: Controls and management for sustainable ecosystem functioning. Oxford University Press, New York, New York, USA.

Peyrard, C., Mary, B., Perrin, P., Véricel, G., Gréhan, E., Justes, E., Léonard, J., 2016. N2O emissions of low input cropping systems as affected by legume and cover crops use. Agriculture, Ecosystems & Environment 224, 145–156. doi:10.1016/j.agee.2016.03.028

Powell, J.R., Welsh, A., Hallin, S., 2015. Microbial functional diversity enhances predictive models linking environmental parameters to ecosystem properties. Ecology 96, 1985–1993. doi:10.1890/14-1127.1

R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Robertson, G.P., Groffman, P.M., 2015. Nitrogen Transformations, in: Soil Microbiology, Ecology and Biochemistry (Fourth Edition). Academic Press, Boston, pp. 421–446.

Robertson, G.P., Hamilton, S.K., 2015. Long-term ecological research in agricultural landscapes at the Kellogg Biological Station LTER site: conceptual and experimental framework, in: The Ecology of Agricultural Landscapes: Long-Term Research on the Path to Sustainability. Oxford University Press, New York, New York, USA, pp. 1–32.

Robertson, G.P., Paul, E.A., Harwood, R.R., 2000. Greenhouse Gases in Intensive Agriculture: Contributions of Individual Gases to the Radiative Forcing of the Atmosphere. Science 289, 1922–1925. doi:10.1126/science.289.5486.1922

Robertson, P.G., Vitousek, P.M., 2009. Nitrogen in Agriculture: Balancing the Cost of an Essential Resource. Annual Review of Environment and Resources 34, 97–125. doi:10.1146/annurev.environ.032108.105046

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Horn, D.J.V., Weber, C.F., 2009. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Appl. Environ. Microbiol. 75, 7537–7541. doi:10.1128/AEM.01541-09

Shcherbak, I., Millar, N., Robertson, G.P., 2014. Global metaanalysis of the nonlinear response of soil nitrous oxide (N2O) emissions to fertilizer nitrogen. PNAS 111, 9199–9204. doi:10.1073/pnas.1322434111

Shoun, H., Kim, D.-H., Uchiyama, H., Sugiyama, J., 1992. Denitrification by fungi. FEMS Microbiology Letters 94, 277–281.

Sinsabaugh, R.., Reynolds, H., Long, T.., 2000. Rapid assay for amidohydrolase (urease) activity in environmental samples. Soil Biology and Biochemistry 32, 2095–2097. doi:10.1016/S0038-0717(00)00102-4

Smith, D.R., Hernandez-Ramirez, G., Armstrong, S.D., Bucholtz, D.L., Stott, D.E., 2011. Fertilizer and Tillage Management Impacts on Non-Carbon-Dioxide Greenhouse Gas Emissions. Soil Science Society of America Journal 75, 1070–1082. doi:10.2136/sssaj2009.0354

Sogin, M.L., Morrison, H.G., Huber, J.A., Welch, D.M., Huse, S.M., Neal, P.R., Arrieta, J.M., Herndl, G.J., 2006. Microbial diversity in the deep sea and the underexplored “rare biosphere.” PNAS 103, 12115–12120. doi:10.1073/pnas.0605127103

Stehfest, E., Bouwman, L., 2006. N2O and NO emission from agricultural fields and soils under natural vegetation: summarizing available measurement data and modeling of global annual emissions. Nutr Cycl Agroecosyst 74, 207–228. doi:10.1007/s10705-006-9000-7

Syswerda, S.P., Corbin, A.T., Mokma, D.L., Kravchenko, A.N., Robertson, G.P., 2011. Agricultural Management and Soil Carbon Storage in Surface vs. Deep Layers. Soil Science Society of America Journal 75, 92–101. doi:10.2136/sssaj2009.0414

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution 30, 2725–2729.

Tiemann, L.K., Grandy, A.S., Atkinson, E.E., Marin-Spiotta, E., McDaniel, M.D., 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. Ecol Lett 18, 761–771. doi:10.1111/ele.12453

Tilman, D., Reich, P.B., Knops, J., Wedin, D., Mielke, T., Lehman, C., 2001. Diversity and Productivity in a Long-Term Grassland Experiment. Science 294, 843–845. doi:10.1126/science.1060391

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

Vandermeer, J., van Noordwijk, M., Anderson, J., Ong, C., Perfecto, I., 1998. Global change and multi-species agroecosystems: Concepts and issues. Agriculture, Ecosystems & Environment 67, 1–22. doi:10.1016/S0167-8809(97)00150-3

Verma, A., Tyagi, L., Yadav, S., Singh, S.N., 2006. Temporal changes in N2O efflux from cropped and fallow agricultural fields. Agriculture, Ecosystems & Environment 116, 209–215. doi:10.1016/j.agee.2006.02.005

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. American Journal of Alternative Agriculture 18, 3–17. doi:10.1079/AJAA200228

Wessen, E., Soderstrom, M., Stenberg, M., Bru, D., Hellman, M., Welsh, A., Thomsen, F., Klemedtson, L., Philippot, L., Hallin, S., 2011. Spatial distribution of ammonia-oxidizing bacteria and archaea across a 44-hectare farm related to ecosystem functioning. ISME J 5, 1213–1225.

Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant Diversity, Soil Microbial Communities, and Ecosystem Function: Are There Any Links? Ecology 84, 2042–2050. doi:10.1890/02-0433

Zhu, X., Burger, M., Doane, T.A., Horwath, W.R., 2013. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N2O and NO under low oxygen availability. PNAS 110, 6328–6333. doi:10.1073/pnas.1219993110

Zumft, W.G., Cárdenas, J., 1979. The inorganic biochemistry of nitrogen bioenergetic processes. Naturwissenschaften 66, 81–88. doi:10.1007/BF00373498

Zuur, A.F., Ieno, E.N., Elphick, C.S., 2010. A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1, 3–14. doi:10.1111/j.2041-210X.2009.00001.x

CHAPTER 5: ALIGNING SOIL HEALTH TESTING AND FARMER KNOWLEDGE FOR IMPROVED ON-FARM SOIL MANAGEMENT

ABSTRACT

The advent of soil health as a framework for management of row crops in the U.S. Midwest has major implications for on-farm agronomic practices. A soil health approach posits a new set of priorities for assessing soil quality compared to traditional forms of soil testing and resultant management recommendations. Here I link farmer knowledge with results from soil testing on their farms, including an emerging approach to test soil health. On grain farms in three Michigan counties with contrasting soil types, I compare results from traditional field crops soil tests with results from soil health tests. For testing, farmers were asked to select their "Best" and "Worst" row crop fields, a "Choice" field of their own interest, and a non-row crop field, such as a wood lot. I held interviews to discuss how soil test results aligned with farmers' own experience of individual fields, how soil testing informs their approach to soil management, and how new approaches to soil testing might alter their practices. Results from traditional soil tests showed no significant differences between comparisons of paired cropped fields for parameters such as pH, phosphorus, potassium or nitrogen. In contrast, soil health test results revealed a large range of differences between field types, in which parameters such as labile soil organic matter and soil aggregation were significantly higher in 'Best" compared to "Worst" fields. Soil health test results also better aligned with how farmers described individual field performance, such as how soils drained or 'worked' physically. While farmers discussed using traditional soil tests primarily for informing fertilizer application rates and monitoring soil pH, soil health test results elicited distinctly broader discussions of soil management considerations, including tillage and crop rotation practices. The results showed that soil health tests can reveal important

differences across farmer fields and suggest both strong opportunities for and notable deficiencies in aligning farmer field knowledge and soil testing to influence soil management decisions that can achieve both socioeconomic and environmental objectives on farms.

INTRODUCTION

The environmental and social costs of industrial-scale agricultural production in the U.S. have led to calls for a transformation to more ecologically-based approaches to management (Drinkwater and Snapp, 2007; Robertson et al., 2014; Schipanski et al., 2016; Shennan, 2008). Soil fertility management is a key dimension of agriculture that has far-reaching impacts for both farm productivity and potential environmental degradation. For example, widespread use of synthetic fertilizers is linked to greenhouse gas emissions and to more than 400 hypoxic zones worldwide (Diaz and Rosenberg, 2008). The Great Lakes region, in particular, has experienced recent severe water contamination and acute drinking water shortages primarily due to upstream agricultural practices (Michalak et al., 2013). While a great deal of attention has focused on opportunities to mitigate the downstream effects of agriculture by providing incentives for farmers to practice alternate management strategies (Cherry et al., 2008), there remains a need to understand how farmers think about and manage for soil fertility. Understanding this decision-making process is critical to inform how a broader range of practitioners could shift toward ecologically-based soil management. Two key entry points directly tied to such a transformation are: i) the quality of soil testing metrics used to assess and manage agricultural soils; and, ii) the extent to which farmers rely on soil tests for management decisions, along with an understanding of the broader frameworks that inform their soil management practices.

Promotion of ecosystem-based approaches to agricultural management has taken various forms, especially in government programs designed to incentivize adoption of conservation

practices (NRCS-CEAP, 2011). However, altering landscape management to mitigate downstream impacts from agriculture by coordinating with farmers to adapt to or confront prevailing socio-ecological conditions poses considerable challenges (Allison and Hobbs, 2004; Wolf and Allen, 1995), especially when soil fertility is so closely associated with crop productivity. One potential leverage point for changing longstanding approaches to soil management is the shift currently underway in soil testing—from a reductionist emphasis on managing for individual inputs/deficits of key plant nutrients, towards a more holistic ecosystem management approach, often termed soil health (Karlen et al., 2008).

Traditionally, soil testing has focused on the supply of plant macronutrients such as nitrogen (N), phosphorus (P), and potassium (K), which Liebig's law of the minimum – formulated in the mid-nineteenth century – posits as key to limiting plant growth if insufficiently supplied in soil. Along with manipulation of soil pH (to optimize nutrient availability to plants), this framework of supplying plant nutrients via inputs to soil has largely guided soil testing and fertility management up to the present (Havlin et al., 2005). Soil tests for field crops (e.g. corn) return recommendations for altering soil pH, if needed, and for fertilizer application rates (particularly for N) for achieving a target yield for a given crop.

Soil health (synonymous with soil quality) incorporates nutrient management into a broader perspective defined as "the capacity of a soil to function, within ecosystem and land use boundaries, to sustain productivity, maintain environmental quality, and promote plant and animal health," (Doran and Parkin, 1994). The multidimensional characteristics of soil health include retaining and cycling nutrients, improved water infiltration and storage, suppression of soil-borne pathogens, better stand establishment, and buffering against damage from drought stress or heavy rain (Moebius-Clune et al., 2016), which are all dependent on agronomic

approaches such as tillage and crop rotation practices. Assessing soil health is most commonly broken into three areas: chemical components such as soil pH and available nutrients, physical components such as soil structure and water holding capacity, and biological components such as the ability to alternately store or release soil carbon (C) and N. Most of the parameters in soil health tests focus on economical or rapid measures of these soil ecosystem properties and processes, and likewise, the recommendations that follow from soil health assessments are meant to foster management practices that enhance these soil functions.

Traditional soil tests have generated decades of fine scale data, such as fertilizer rate recommendations for specific crops in specific counties on specific soil types. In contrast, soil health tests are still in the early stages of deployment and dissemination as information resources for farmers. To support their effective implementation on a broad scale, they need to be vigorously tested on farmer fields in a broad range of management and environmental contexts. Even though many studies have demonstrated that soil health parameters are sensitive to changes in soil management (Moebius-Clune et al., 2008), this may not ensure that soil health tests capture soil quality differences on farm fields, or in distinct regions, in ways that correspond to or are relevant to farmer experience. Many soil health testing parameters have proven effective for assessing differences in soil quality in long-term experimental gradients on research sites (Bhardwaj et al., 2011; Obade and Lal, 2016) but ultimately they must be able to reveal differences on farmer fields on time-scales that can inform farm management decisions. Compared to traditional soil tests that primarily recommend fertilizer and lime input rates, farmer decisions based on soil health test output may extend into a broader suite of cropping system practices, such as tillage, residue management, or crop rotation including use of cover crops. Thus, it is critical that soil health tests provide accurate, field-specific data on which farmers can

rely for these decisions. Finally, even if soil health tests accurately capture soil biological, physical, and chemical variability on farmer fields, ultimately how farmers conceptualize soil quality will influence how they interpret and implement agronomic decisions based on test results. While traditional soil tests directly link plant nutrient inputs to crop yield within a growing season, addressing soil health problems – which may also severely impact crop performance – may take careful planning over several seasons to produce a desired outcome. A soil health management framework must therefore accurately reflect conditions on farmer fields, effectively guide soil management decisions, and lead to positive agronomic performance for farmers while minimizing impacts on surrounding ecosystems.

In this study I aimed to integrate technical and social dimensions to understand how to adapt soil health testing to farms. Specifically our research addresses three overarching objectives to inform soil health testing on Michigan farms: i) to evaluate how soil health tests capture management variability on farmer fields, ii) to understand what information, including soil testing, farmers consider when making soil management decisions, and iii) to assess how farmers understand soil quality and respond to soil health test results in comparison to traditional soil testing from their own fields. Using a targeted approach by asking MSU Extension staff to identify farmers interested in soil testing, I conducted an in-depth, mixed-methods study in partnership with 13 row crop farmers in three geographically distinct regions of Michigan.

METHODS

Experimental approach

MSU extension and staff from each region contacted farmers who would be willing to be interviewed in exchange for soil testing on four distinct field types. I asked participant farmers

to identify four fields to sample for soil health testing based on their judgment of a 'Best' and 'Worst' field, an additional 'Choice' field, which they wished to test, and a non-row crop (NRC) field, such as a woodlot or hay field. Through this approach I captured a wide range of field variability as identified by growers on which to test and compare soil health parameters. It also provided a participatory approach as a means to engage directly with farmers as decision-makers. I collected soil samples from each of 52 fields across all farms, and measured management-sensitive parameters of soil health to examine how well these tests characterized soils on farm fields, as compared to traditional field crop tests offered by Michigan State University (MSU). I then conducted in-depth interviews with growers to evaluate how well soil health results reflected the management histories for each field type, and to understand their own assessments of soil quality and using soil testing, and how these inform their soil management decisions and cropping practices. My approach presumes that managing for soil health on farms derives from farmer knowledge of and practices on their own fields; from scientists' capacity to accurately assess and communicate soil health testing results; and, from feedbacks between these two types of knowledge that may lead to emergence of a soil health management framework.

Field sampling

I sampled farms in three Michigan counties: Presque Isle (45°42'N, 83°81'W), Isabella (43°60'N, 84°76'W), and Van Buren (42°21'N, 85°89'W), representing north, central and southwestern regions, which have distinct climates and geography. I visited each farmer and briefly discussed the history of the four differently managed fields they selected: 'Best', 'Worst' fields, NRC, and 'Choice'. At each field I noted soil conditions and recorded crop management observations, and followed the Cornell Soil Health Assessment approach (Moebius-Clune et al., 2016) when taking soil samples. I selected five locations on each field representative of each

management designation, and used a shovel to made a small pit, a 'slice' of bulk soil from the side of the pit, 15 cm depth, and approximately 4 cm thick and 9 cm wide. At each sampling point in the field, two separate penetrometer (Soil Compaction Tester 6120, Spectrum Technologies) readings were taken at 15 cm and 45 cm depths. All field samples were mixed thoroughly in the field and a sub-sample (approximately 2 kg) was stored in a plastic bag, and placed on ice until further processing. All samples were collected from May to June of 2014, on thirteen farms for a total of 52 fields, and maintained at 4° C until processing.

Soil testing

As soon as possible after return from farmer fields, samples were sieved to < 8mm to remove stones and a homogenized sub-sample was submitted to the MSU Plant, Soil and Nutrient Laboratory (SPNL) for analysis of pH, total soil organic matter (SOM) by loss on ignition, total Bray 1 phosphorus (P), potassium (K^+), Calcium (Ca^{+2}), Magnesium (Mg^+) as well as ammonium (NH_4^+) and nitrate (NO_3^-). We grouped these parameters into 'chemical' results except for SOM. At the Kellogg Biological Station I carried out soil health analysis for soil physical parameters (soil texture, aggregate stability, and available water capacity) and soil biological factors (permanganate oxidizable carbon, potentially mineralizable carbon, potentially mineralizable nitrogen, nitrification potential).

Soil texture was determined following Kettler et al. (2001) on soil sieved to < 2mm and dried overnight at 60° C. A 14 g portion of this soil was placed into 50 ml Falcon tubes with 42 mL of 3% hexametaphosphate solution and tubes were placed on their side on a shaker at 120 rpm for two hours. Contents of each tube were poured through a 0.053mm sieve and thoroughly washed with 600 mL deionized water, which was caught in a basin. The sand fraction on the sieve was washed into a previously tarred drying can. Particles in the catch basin were

thoroughly re-suspended and allowed to settle form 4-6 hours, after which the clay particles in suspension are decanted and settled silt particles are washed into another tarred drying can. All cans were dried overnight at 105° C and contents weighed. Texture was calculated as: Sand % = (oven dry sand mass / original sample mass) x 100%; Silt % = (oven dry silt mass / original sample mass) x 100%; Clay % = 100 - (Sand % + Silt %).

Wet aggregate stability (AS) was determined following Moebius et al. (2007) from soil dried to constant weight at 40° C (1-2 days in the oven) followed by isolation of aggregate size fraction 0.25mm-2mm. Ten g of aggregates were spread evenly on a 0.25mm mesh, 125 mm diameter sieve. The sieve was placed on a funnel containing a previously weighed filter paper and the funnel with sieve was placed on a ring stand. Sieves were exposed to a rain simulator (rate previously calibrated) for five minutes, after which any the material retained on the sieve was thoroughly washed through and remaining particles (e.g. small stones) were washed off the sieve surface into a drying can. The can and filter paper with slaked soil were dried for 1 day at 100° C oven, and AS was calculated as the percentage of soil retained on the sieve (difference from what was not slaked onto the filter), and adjusting for the mass of un-sieved particles.

Available water capacity (AWC) was determined following Reynolds and Topp (2008), with a portion of soil dried to 60° C was sieved to < 2 mm. Two 15 g portions were spread evenly inside brass rings and placed on ceramic plates with known porosity, and saturated with water. Plates were placed into high pressure chambers, one set to 10 kPa (to determine field capacity) and the other set to 1500 kPa (permanent wilting point). After samples equilibrated inside each chamber, soils were removed and weighed, dried at 105° C and then weighed again. AWC was calculated as soil water loss between samples at 10 and 1500 kPa, and reported as g water per g soil.

To determine labile carbon as permanganate oxidizable carbon (POXC), air-dried soil was used according to Weil et al. (2003) . Duplicate 2.5 g samples of dried soil were mixed with buffered 0.02 M $KMnO_4$ solution in 50ml conical tubes, shaken at 120 rpm for two minutes and allowed to settle for eight minutes. From this reaction, 0.5 mL of supernatant were diluted with 49.5 mL of deionized water. The degree of oxidation was measured colorimetrically at 550 nm on a Fisher Scientific Thermo Multiskan microplate reader (Waltham, MA.) and standardized to a series of known $KMnO_4$ standards.

Air-dried soil was also used to determine potentially mineralizable carbon (PMC) according to Franzluebbers et al. (2000). For each field, replicate ten g of soil were placed in Mason jars and brought to 50% water-filled pore-space and incubated at 25^o C. Following the 24-hour incubation, Mason jars were capped tightly and at time zero a CO_2 reading was taken immediately, by injecting 0.5 mL of headspace into a LI-COR LI-820 infrared gas absorption analyzer (LI-COR Biosciences, Lincoln, NE). Three subsequent readings were taken over 90 minutes and a flux was calculated by regressing the change in CO_2 against incubation period (Robertson et al., 1999).

Potentially mineralizable N (PMN) was determined from fresh soil sieved to < 2mm and incubated anaerobically following (Drinkwater et al., 1996). From duplicate 8 g soil samples, NH_4^+ and NO_3^- were extracted with 1M KCl on a rotary shaker at 120 rpm for 1 hour. To two additional 8 g replicates soil samples in conical tubes, 10 mL deionized water was added and dinitrogen gas was used to replace tube headspace air and bubbled into the slurry for one minute prior to sealing with butyl rubber stoppers. Sealed tubes were incubated at 25 °C for seven days. After seven days the stoppers were removed, buffer was added to bring the slurry to 1M KCl, and samples were shaken, filtered, and stored in the same manner as fresh samples.

Concentrations of NH_4^+ were determined colorimetrically at 630 nm (Sinsabaugh et al., 2000), and NO_3^- at 540 nm following Doane and Horwath (2003). PMN was determined from the concentration of NH_4^+ from incubated soil minus NH_4^+ from initial soil extraction.

Nitrification potential (NIT) was determined using fresh soil sieved < 2 mm and following Norton and Stark (2011). Fifteen g soil was added to a solution with 1.0 mM NH_4SO_4 and 1mM phosphate (dilute mixture of 1.5 M KH_2PO_4 and 3.5 ml 0.2 M K_2HPO_4 in 1 L) in a flask, loosely capped, and placed on a shake at 200 rpm. Over the next 24 hours, 1.5 mL samples were taken from the slurry at four time points (2, 4, 18, 24 hours), placed in a microfuge tube and spun down at 8000 x g for 8 minutes. Supernatant was decanted and stored at -20° C for later analysis for NH_4^+ as described above. Potential was calculated by regressing the change in NH_4^+ against incubation period time points.

Statistical approach

All soil data analyses were calculated in R (R Core Team, 2016). For all soil testing parameters, paired t-tests were carried based on field type comparisons (e.g. Best compared to Worst) across all farms.

Farmer interviews

After compiling all soil testing data, I held interviews, divided into three components, with all participant growers. In the first component, I discussed the management history of each field, including crop rotation, tillage, how the farmer had chosen specific management decisions for each field, and why the farmer had assigned the given field to each category (i.e., 'Best' and 'Worst'). In the second component, I discussed specific test results for all fields, grouping results into physical, biological, and chemical categories. During the third component, I

facilitated an open discussion of soil test results and the factors that influenced management decisions for specific fields. These open discussions were aimed at integrating soil test results with farmer knowledge/experience to summarize how well the test metrics related to farmer interpretation of soil management for each field type. All of the interviews were recorded, and notes were written up within 24 hours of each interview. Recordings were transcribed and analyzed for common themes related to each soil test parameter; different approaches to soil management based on each of the field types; and, on the influence of soil testing on management practices.

Using written notes and transcripts, the component interviews with each farmer were analyzed based on three groupings. First, answers to core questions, which were asked of all participants, were compiled and summarized into concise response summaries. Second, we grouped farmer responses to the brief discussions of individual soil test results for each field type by test result, and summarized findings for each soil test metric. Third was an analysis of open discussions about testing interpretation and management for each grower on each field type. These discussions also identified which results the farmers found most noteworthy/valuable, and how they overlapped with their experience of management from each field type. For this third section, farmers' reactions to testing based on their experience of each field and its history of management were noted and key quotes extracted.

RESULTS

Comparing soil tests parameters across field types

Comparing physical parameters across Best and Worst fields, both AS and AWC differed significantly between paired fields on each farm (Table 5.2). Conversely, measures of surface and sub-surface hardness did not differ between these field types. Surface and subsurface

compaction fluctuated with the status of the field on the day of sampling (Table 5.2). Different fields had been tilled or prepared to differing degrees, or had variable amounts of residue or cover which did not necessarily vary by the grower's field type designations.

Three of the biological parameters PMN, POXC and NIT – did not show significant differences between Best and Worst fields (Table 5.3). Percent SOM and PMC were both significantly higher in the Best fields. None of the chemical data measured in the traditional field crops test (pH, P, K^+, Ca^{+2}, Mg^+, NH_4^+ and NO_3^-) showed any significant difference between Best and Worst fields (Table 5.4).

The non-row crop (NRC) fields ranged greatly in their management and land-use histories, from woodlot to hay field to buffer strip. However, all had been under some kind of perennial cover for at least the previous five years (Table 5.5). In terms of the physical parameters, only surface compaction was significantly higher on NRC (Table 5.2). Paired comparisons of Best and NRC fields indicated that SOM, POXC and PMN were all significantly higher on the Best field, and NIT was significantly lower on the NRC field (Table 5.3). Again, none of the soil chemical parameters were different across these field type comparisons (Table 5.4). A similar set of results emerged when comparing Choice and NRC, in which OM, POXC, PMN, and NIT were all more favorable for the latter field. The contrast between NRC and Worst field was even greater, with the former having significantly higher AWC, OM, POXC, PMC and PMN. The NRC fields were also higher in Ca^{+2} than the Worst field.

Farmer interviews on soil health test results

All farmers in the study conducted soil samples every 2-4 years for a given field (Table 5.6). Most of them use this information directly to determine their fertilizer and lime application rates, rather than to provide indicators of soil quality. Two of the farmers also used soil tests

when considering controlled traffic (tractor passes) on fields, and two others used them to keep track of excess P additions. In contrast, farmers primarily assessed *soil quality* as a physical characteristic: how the soil 'works' (when planting and tilling), whether or not the surface crusts, how a field drains, etc. Other indicators such as organic matter content, lack of soil-borne disease, and the importance of longer crop rotations were also discussed by a minority of farmers when asked to describe soil quality.

In describing how they make decisions about soil management practices, a majority of farmer participants mentioned the role of social networks (e.g., family and neighbors), and the importance of on-farm experimentation with different practices—either directly on their fields, or observed on other's fields. A minority of farmers also mentioned relying on information from soil testing agencies, sales representatives, and on literature from extension agents, trade magazines, or the World Wide Web as key knowledge resources.

Nearly all of the farmers designated the Best and Worst field types based on their experience of yields from each field, as well as the contrasting predictability of management outcomes between the two fields (Table 5.7). Best fields were more reliable, but also appeared to receive more management attention (i.e., they were cared for more closely). Aside from yield, farmers described Worst field characteristics in physical terms, as 'working' poorly or having problems with too much or too little moisture. Finally, Choice fields were selected based on yield concerns, but also due to more specific concerns, such as compaction or uncertainties over prior management (Table 5.7).

Farmers responded differently to soil testing results depending on the parameter measured for each field type. Those who responded to soil physical measures (e.g. AS, AWC) did so with their explicit experience of a particular field, such as describing how the field

typically drains or 'works' (Table 5.9). For example, one grower responded to the contrasting values for AWC on his Best and Worst field types by describing how the fields respond to rain. He noted that in his Best field " the water hits it and it absorbs it," whereas for his Worst field, "when water hits off this one it beats it, and then hits a table, and then just has to evaporate off…then the sun bakes it and you got clay."

For SOM, most farmers were aware of the SOM levels on each cropped field type, but their interest in altering this value depended on the region. Referring to increasing SOM values on his fields, one farmer in Isabella County – who farms a finer-textured soil – remarked, "I am not going to live long enough to do that." By contrast, in counties with coarser soils (Van Buren and Presque Isle) farmer were more interested in how increase organic matter through changes in management. A majority of farmers responded that they would like to be able to monitor shorter-term changes in soil C (such as PMC) if they could reliably do so. On most farms, C parameters and physical characteristics showed clear contrasts across the fields of interest. Measures of PMN and NIT did not, and farmers had fewer responses to these parameters. Finally, for soil chemical parameters, farmers responded most strongly to values for pH and P across different field types, as these are parameters they actively manage through soil testing.

DISCUSSION

Comparing soil testing approaches

Soil health tests reflected greater sensitivity to soil parameters across four different field types than the traditional field crops test. Only total SOM, designated as a biological parameter in this study, and assayed as part of the field crops test was significantly different between NRC and all cropped fields (Best, Worst, Choice) as well as between Best and Worst. For the same set of field comparisons, one or both soil health estimates of labile soil carbon, POXC and PMC,

also showed significant differences. By contrast, with the traditional field crops test, none of the field comparisons revealed any significant difference in soil pH, NH_4^+ or NO_3^-. Neither were other plant nutrients in the field crops test (P, K^+, Mg^+) significantly different between paired field comparisons, except for Ca^{+2}, which had a lower concentration in Worst and Choice fields. Indeed, of the 52 fields measured for P, K^+, Ca^{+2}, and Mg^+ (204 separate results), only ten results rated below 'Optimum' concentration, as designated by the MSU Soil Testing Laboratory (http://www.spnl.msu.edu/). The majority of these ratings were for K^+, and included fields from all four of the field types selected by participant growers.

Meanwhile, soil health test parameters captured differences in other management-sensitive indicators between field types. Soil aggregation and water holding capacity, positive indicators of soil physical health, were higher in Best fields with AS and AWC significantly greater compared to Worst fields. For indicators of soil N, mineralizable N and nitrification were different in NRC fields, with PMN significantly higher in NRC fields when compared to all other cropped field types, and NIT lower in NRC fields, both of which might be expected in an uncropped field. However, among comparisons of cropped fields, soil health assays for labile soil C and soil physical health showed clear differences between crop fields of interest to farmers, especially when compared to traditional field crops tests.

How farmers make soil management decisions

The targeted approach of this survey meant that many of the farmer participants had been identified by MSU Extension staff as careful and even exceptional managers for their region. In terms of soil test metrics, pH was the only one that farmers actively managed on a longer time scale. Farmers care about SOM, but indicated that they don't generally manage for it. This is

likely because they receive the greatest financial incentives to intensify and manage for high yields, using plant nutrient inputs, rather than to diversify crop rotations or adopt other ecological practices that are likely to build SOM (even if that might ultimately feedback to increase productivity with reduced input costs in the long term). By and large these farmers were innovators and spoke in terms of having tried, or recognized the need to try, new approaches to soil management. Most of them want to improve their cropping practices on specific fields and were willing to make changes. That being understood, farmers knew that improving soil quality can mean large changes in management, such as altered crop rotations, but have few guideposts to point them in the right management direction.

Aligning soil quality with soil testing

Using farmer knowledge to sample a range of field types made it possible to assess how well different soil testing parameters capture soil variability as characterized by farmers. Traditional soil test parameters did not reflect the strong differences in productivity across these field types, though farmers are already actively managing based on these testing parameters (e.g. pH and P). Indeed, the farmers in this study were purposefully selected and agreed to participate because of an expressed interest in soil testing. Soil quality, in contrast, is more intuitively understood through farmers' own experience and on-farm experimentation – they know it when they see it and feel it. Farmers frequently described soil quality in physical terms for which soil health parameters (AWC and AS) tended to overlap with their experience of managing specific field types. Farmers also knew that managing to build organic matter improves soil quality and expressed interest in knowing how soil health measures of labile soil C (POXC, PMC) could give them greater management control over this resource.

Following a review of soil health results on their farm, we discussed how they approach soil management across their fields to better understand how a soil health framework intersects with farmer knowledge and practice. I identified three key themes that emerged from this mixed-methods approach that combined knowledge from targeted soil testing with farmer experience.

Emerging themes

I. Soil health metrics need to be solid and demonstrable.

Farmers are not sure how to address problems associated with lower soil health test values. They believe the soil health numbers (e.g. the testing differences on contrasting field types) but they need more specific guidance. For example, they want know *how* to link a specific testing result to a change in practice, and to be able to trust and see that change.

II. Testers need to know how to use the test, and what its limits are.

Some structural constraints conflict with soil health testing. These may include crop prices, or the time scales over which farmers are willing to consider management changes. Educating farmers about soil health tests and their benefits will likely be insufficient to leverage change if the recommendations are not likely to be adopted by farmers because of other constraints. This could mean the need to develop targeted tests (e.g., perhaps starting with a full assessment, with follow-up testing focusing on one or two critical metrics). Soil health tests may be superior to traditional tests for informing more sustainable management, and, in the near-term they are clearly important for on-going research on soil and cropping system management. In the long-term, if changing socioeconomic conditions favor a greater number of sustainable farming practices, these tests will be ideal tools for extension and technical assistance to inform

management goals on a broader range of farms. In either case, soil health tests are not useful for farmers unless they target practices that farmers are willing to undertake, especially if the test helps to motivate on-farm changes in management.

III. Soil health tests need to adapt to regional differences in soil and farm practices.

Different soil types and environmental conditions dictate both different management styles and farmer responses to soil health metrics. For example, farmers on 'heavier' ground care less about organic matter because it takes more effort to increase this resource. Instead, they care more about soil structure (i.e., the aggregate stability test). Farmers on lighter, coarser soils care more about how to improve their organic matter – they know they can change it from their experience (not from following soil testing results) and from seeing the difference in improved soil performance. Some test results may show poor numbers that are characteristic of regional patterns. Similar to traditional testing, soil health tests need minimum data sets and databases to strengthen their predictive ability and relevance to specific soil types and environmental contexts.

Synthesis

Soil health testing revealed strong differences across farmer fields, particularly for values related to soil organic matter and physical factors. Perhaps more importantly, these tests distinguished key soil parameters between field types, which farmers had selected as either good or problematic fields. The NRC field also proved useful in highlighting to farmers differences in cropped fields, showing the greatest contrast in soil health measures of water holding capacity, and pools of soil C and N. Further, the NRC field was the only field to show differences in

nitrification potential compared to other fields, highlighting the opportunity to further adapt testing parameters to other management sensitive ecosystem processes. Michigan has a highly diverse agricultural sector and to our knowledge, extensive soil health surveys have not been conducted across different regions and agroecosystems. There is a critical need to establish baseline surveys and build a database (which already exists for traditional soil tests), in order to strengthen confidence in soil health testing results and recommendations.

Farmers rely on many different types of knowledge to make decisions about adjustments to their soil management, including their own experience and competencies, and information from outside sources such as crop advisers, extension agents, and soil testing agencies. In turn, soil management decisions, are impacted by a complex set of social and ecological factors. Adoption of soil management recommendations (such as might come from a soil health assessment) may depend on the size and scale of the farm (Stuart and Gillon, 2013), the financial capacity of the farmer (Singer et al., 2007), a farmer's access to information and networks (Baumgart-Getz et al., 2012), their trust in different sources of information (Wolf and Nowak, 1995), or on their willingness to engage with and seek out innovation (Blesh and Wolf, 2014). Given the many factors that may influence a farmer's decision about soil management, it is critical that results from soil health tests intersect with farmers' assessments of their own fields so management recommendations can be aligned with farmer knowledge.

A framework for managing from soil health tests must not only reflect field conditions but recognize that farmers have clear ideas about soil quality (Romig et al., 1995; Walter et al., 1997), which inform how they manage fields and how they understand the effects of changing practices. For example, my results suggest that farmers readily linked results from soil physical measurements to their own observations in the field such as soil crusting, compaction, or field

drainage, and were more interested addressing these results through changing their practices. Yet, even in this targeted sample of farmers, regional differences influenced soil testing results and farmer interpretation. All farmers expressed interest in increasing organic matter, but the weight placed on this value depended on the region. While traditional soil tests link plant nutrient inputs directly to yield, findings from soil health tests, which reflect severe limits to crop performance, such as poor soil aggregation or low labile organic C, may require a more complex set of management decisions. Thus, changes in soil management may also depend on planning and long-term horizons (Lambert et al., 2006), such as a farmer's willingness to discount cost or risk in the short-term in order to reach a longer term goal or benefit. Based on clear differences in results across their fields, nearly all farmers in this study expressed interest in continuing soil health testing, but wanted a better understanding of how specific management practices, under their control, linked to positive testing outcomes and improved soil quality.

Conclusions

As private landowners, farmers have the greatest incentive to manage their fields for near-term goals such as yield or profitability. Yet, a corollary to a focus on soil health management is that agricultural landscapes are increasingly expected to supply a range of ecosystem services beyond yield—including non-provisioning services (Dobbs and Pretty, 2004; Robertson et al., 2014). Even though policy and market conditions often limit transitions toward sustainability (Stuart and Gillon, 2013), a growing number of farmers are explicitly managing for soil quality. However farmers lack soil testing information that may help guide management toward soil quality. Results from this study suggest that soil health tests can detect differences in a range of soil quality parameters across working farms. Since these parameters also intersect

with farmer knowledge of their fields, they can potentially inform changes in management. All farmers in this study use information from traditional soil tests to inform critical and costly management decisions such as fertilizer and lime applications. They also use other information when deciding how to manage their soil, including information from networks of family and neighbors, and from their own experimentation. When considering significant changes to soil management that might follow from soil health test recommendations, these farmers expressed concerns related to factors such as financial risks and availability of time, which have also been identified in other farmer surveys (Baumgart-Getz et al., 2012). Using soil health testing in ways that target important soil parameters and track progress from recommended management practices, farmers may be more willing to adopt changes that have advantages to soil quality over the long term (Chouinard et al., 2008). Likewise, knowledge from on-farm research and understanding of farmer practice will inform the activities of soil researchers. Managed ecosystems are dynamic, complex systems, and the need to integrate models of human behavior and decisions with biophysical measures is critical to improve environmental outcomes (Antle et al., 2001). Expanding soil health testing must include careful work with farmers in order to integrate an ecosystem-based framework for managing soil.

AKNOWLEDGEMENTS

Support for farmer surveys and testing were supported by a the U.S. Department of Agriculture's Sustainable Agriculture Research and Education program, grant # GNC14-192, and also by a summer fellowship from the Kellogg Biological Station's Long-term Ecological Research Program through the National Science Foundation (DEB 1027253).

APPENDIX

Table 5.1 Percent sand and soil texture class from 13 farms in three Michigan counties for four field types: "Best Field" and "Worst Field" as characterized by farmers, a non-row crop field ("NRC") and a "Choice" field also selected by each farmer.

	Best Field		Worst field	
County	% Sand	Texture class	% Sand	Texture class
Van Buren	81.6	Loamy sand	82.6	Loamy sand
Van Buren	64.8	Sandy loam	62.8	Sandy loam
Van Buren	85.3	Loamy sand	92.3	Sand
Van Buren	85.0	Loamy sand	81.1	Loamy sand
Presque Isle	87.9	Loamy sand	82.1	Loamy sand
Presque Isle	81.5	Loamy sand	81.0	Loamy sand
Presque Isle	73.6	Sandy loam	64.2	Sandy loam
Presque Isle	70.0	Sandy loam	71.4	Sandy loam
Isabella	41.6	Medium loam	37.6	Medium loam
Isabella	62.5	Sandy loam	71.0	Sandy loam
Isabella	66.1	Sandy loam	79.9	Loamy sand
Isabella	66.3	Sandy loam	64.4	Sandy loam
Isabella	86.1	Loamy sand	71.5	Sandy loam

Table 5.1 (cont'd.)

	NRC		Choice	
County	% Sand	Texture class	% Sand	Texture class
Van Buren	83.2	Loamy sand	89.5	Loamy sand
Van Buren	65.2	Sandy Loam	69.0	Sandy loam
Van Buren	85.8	Loamy sand	79.0	Loamy sand
Van Buren	85.9	Loamy sand	86.3	Loamy sand
Presque Isle	84.6	Loamy sand	92.1	Sand
Presque Isle	82.8	Loamy sand	90.2	Sand
Presque Isle	81.6	Loamy sand	69.3	Sandy loam
Presque Isle	76.1	Sandy loam	74.3	Sandy loam
Isabella	60.4	Sandy loam	51.4	Medium loam
Isabella	71.4	Sandy Loam	69.4	Medium Loam
Isabella	77.9	Loamy sand	71.1	Sandy loam
Isabella	91.0	Sand	49.4	Medium loam
Isabella	44.9	Medium loam	55.1	Sandy loam

Table 5.2 Percent aggregate stability (AS), available water capacity (AWC), soil compaction measures as resistance at two soil depths shown as means ± se for across Michigan farms (n=13). Field types are "Best Field" and "Worst Field" as characterized by farmers, a non-row crop field ("NRC") and a "Choice" field also selected by each farmer. Field comparisons are t-tests comparing paired field types for each farm for each soil parameter. Significance of treatment effect: * P ≤ 0.05, ** P = 0.01 to 0.001, ***P<0.001.

Field Type	AS	AWC	Resistance at depth	
			0-15 cm	15-46 cm
	%	g water g soil^{-1}	psi	
Best	69.1 ± 4.0	0.20 ± 0.02	15.3 ± 3.6	59.2 ± 3.1
Worst	58.7 ± 5.4	0.15 ± 0.02	20.3 ± 4.2	59.0 ± 4.3
NRC	70.3 ± 5.0	0.24 ± 0.02	30.2 ± 7.5	66.5 ± 6.9
Choice	59.8 ± 4.7	0.19 ± 0.02	25.1 ± 6.3	61.2 ± 5.0

Field comparison	AS	AWC	Resistance	
			0-15 cm	15-46 cm
	t-test	t-test	t-test	t-test
Best vs. Worst	2.4*	2.9*	-1.4	0.0
Best vs. NRC	0.1	-1.3	-2.3*	-1.5
Worst vs. NRC	-1.3	-4.2***	-1.4	-0.7
Worst vs. Choice	0.0	-1.1	-0.7	-0.1
Choice vs. NRC	1.3	1.9	0.7	1.1
Best vs. Choice	1.9	1.7	-2.1	-1.0

Table 5.3 Percent organic matter (OM), permanganate oxidizable carbon (POXC), potentially mineralizable carbon (PMC) and nitrogen (PMN) and nitrification potential (NIT) shown as means ± se for across Michigan farms (n=13). Field types are "Best Field" and "Worst Field" as characterized by farmers, a non-row crop field ("NRC") and a "Choice" field also selected by each farmer. Field comparisons are t-tests comparing paired field types for each farm for each soil parameter. Significance of treatment effect: * $P \leq 0.05$, ** P = 0.01 to 0.001, ***P<0.001.

Field Type	OM	POXC	PMC	PMN	NIT
	%	mg POX-C kg^{-1}	μg CO_2-C g^{-1}	μg NH_4^+-N g^{-1}	mmol NO_3^--N kg^{-1} g^{-1}
Best	3.5 ± 0.3	283 ± 28	39.5 ± 3.4	6.5 ± 0.6	5.2 ± 0.3
Worst	3.1 ± 0.2	287 ± 21	29.2 ± 3.6	6.0 ± 0.6	4.8 ± 0.6
NRC	4.7 ± 0.6	405 ± 26	43.5 ± 4.6	9.2 ± 0.7	3.3 ± 0.4
Choice	3.2 ± 0.2	295 ± 32	30.3 ± 6.3	6.6 ± 0.6	4.8 ± 0.5

Field comparison	OM	POXC	PMC	PMN	NIT
	t-test	t-test	t-test	t-test	t-test
Best vs. Worst	3.5**	-0.2	2.4*	0.6	0.7
Best vs. NRC	-3.0*	-2.8*	-0.6	-2.4*	3.7**
Worst vs. NRC	-3.5***	-3.2***	-3.1**	-2.9*	2.1
Worst vs. Choice	0.1	-0.4	-0.1	-1.0	0.2
Choice vs. NRC	3.0*	2.3*	2.0	2.9*	-2.3*
Best vs. Choice	1.4	-0.2	1.6	-0.2	1.0

Table 5.4 Soil pH, soil concentration Bray1 Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), ammonium (NH_4^+) and nitrate (NO_3^-) shown as means ± se for across Michigan farms (n=13). Field types are "Best Field" and "Worst Field" as characterized by farmers, a non-row crop field ("NRC") and a "Choice" field also selected by each farmer. Field comparisons are t-tests comparing paired field types for each farm for each soil parameter. Significance of treatment effect: * $P \leq 0.05$.

Field Type	pH	P	K	Ca	Mg	NH_4^+	NO_3^-
		ppm	ppm	ppm	ppm	µg NH_4^+-N g^{-1}	µg NO_3-N g^{-1}
Best	6.85 ± 0.18	94.2 ± 18.6	145.2 ± 14.0	1378.6 ± 214.9	185.5 ± 24.4	1.6 ± 0.1	11.9 ± 2.4
Worst	6.85 ± 0.20	82.9 ± 21.4	138.5 ± 15.4	1228.5 ± 153.2	194.3 ± 26.3	3.2 ± 1.6	10.9 ± 2.3
NRC	6.94 ± 0.19	88.6 ± 16.9	153.7 ± 26.9	1658.3 ± 265.6	205.2 ± 33.1	2.5 ± 0.5	11.1 ± 1.6
Choice	6.88 ± 0.17	76.3 ± 13.6	142.9 ± 20.3	1194.4 ± 192.2	173.8 ± 22.7	1.7 ± 0.2	7.5 ± 1.8

Field comparison	pH	P	K	Ca	Mg	NH_4^+	NO_3^-
	t-test	t-test	t-test	t-test	t-test	t-test	t-test
Best vs. Worst	0.3	-0.1	0.4	1.1	0.0	-0.9	0.7
Best vs. NRC	-0.4	0.7	-0.1	-1.1	-1.2	-1.7	0.6
Worst vs. NRC	-0.9	0.3	-0.3	-2.5*	-0.9	0.4	0.1
Worst vs. Choice	-0.4	0.7	0.1	0.1	1.1	1.0	1.8
Choice vs. NRC	0.3	0.8	0.4	2.0	1.5	1.3	1.7
Best vs. Choice	-0.1	1.4	0.3	2.9*	1.1	-0.2	1.6

Table 5.5 Field observations from sampling on 4 field types on 13 farms across Michigan.

Farm Number	Best	Worst
1	Best field is heavily tilled. Appears to have been in soybeans the previous year. Cloddy and crusted. Poor structure but appears to have decent organic matter. Formerly part of swine operation? Poor aggregate structure and largely unconsolidated. No evidence of earthworm or other biological activity. No weeds.	Planted and plowed. Beyond tree line seen from best field. Structure is poor and organic matter appears quite low. Highly unconsolidated and loose – little to no horizon.
2	'High' field. Was in corn with lots of residue remaining. High level of earth worm activity. Field has received a lot of manure since it is closest to the paddock. Planted in soybeans already (no-till) and some prills of fertilizer also visible. Soil is dark. Nice structure. No evidence of drainage problems. Looks in excellent condition. Evidence of mycorrhizae.	'Dad/Bill' field on the other side of the highway. Planted in soybeans. Field has been disked and appears to have been in corn. Evidence of earthworm activity, but looks like poor organic matter and not very good structure.
3	'Dad' field. Soil is clayey but without too much structure. Appears highly compacted and blocky. 'Heavy.' Some oxidation – poor drainage and patchiness.	'Dave Good – west of the white house'. Lots of earth worm activity, and some crusting. Almost nothing growing. Soil highly friable, but decent structure and some blockiness as peds. Coming out of corn with large amount of residue remaining.
4	No-till and modified strip-till. Some areas not tilled in 100 years. Flat and recently planted to soy. Lots of corn residue. Highly structured soil and looks to be in excellent condition. Low-lying but drainage appears good.	'Smith/Low', Compacted. Crusted. Planted to soy last year with moderate amount of residue remaining. Some weediness 'between' rows. Some gleying and possible drainage issues.

Table 5.5 (cont'd)

Farm Number	Choice	Non-row crop
1	Across the road from the best field. Clear drainage problems here – evidence of outwash, rocky and high level of mottling. Some degree of variability across from field too as it leads to trees. Previously in corn. Crusted.	Grassy area need road and by walnut trees. Highly structured and deep horizon. Some sandiness but generally dark with high organic matter. Covered primarily in orchard grass. Highly moist.
2	'Across the way' On the other side of a drainage ditch from the alfalfa field. Field has more structure and less of the drainage problems. Slopey. Planted to corn. Lots of earth worms.	'Foxes-Hay' is coming out of pasture (alfalfa) which appears to have been strayed. Alfalfa looks poor and spotty. Lots of earth worm activity. Field appears to drain poorly also – mottling with glaying and oxidative patches.
3	CRP ground across the way from main farm house and at the corner. Deciduous forest. Sandy with but high organic matter. Appears to be poorly drained. Thick understory. Highly structured.	Sandy and windswept. Rocky at the surface. Field looks 'burned' but weedy – lots of quackgrass. Soil very poorly structured but with mottling – drainage problems (streaky oxidation)
4	Lots of earth worm activity. Appears highly crusted. Planted to no-till and appears to have been kept in no-till for a while. Soil is highly aggregated and well-structured. Appears to drain well.	Grassy strip adjacent to high yield field. In lush grass – very deep horizon and high organic matter. Full or roots and well-structured.

Table 5.5 (cont'd)

Farm Number	Best	Worst
5	Some crusting but rich-looking and dark with lots of evidence of worm castings. Sandy and does not hold together well. Across and further down the road from Dandelion buffer strip. 'High-R17 and R15'. Would appear to have received a lot of manure. Near lots of old out-buildings.	Previously planted to Sorghum (this is the filed that took an age to find – last one of the day.) Crusty with lots of weeds. 'Kelly-Meyers'. Coming out of sorghum. Moderate amount of residue. Lots of weeds. Lots of crusting. Field is highly sloped and has some drainage problems at one end.
6	Chris - Soybean, lots of corn litter. Very hilly. Some weeds. Dark sandy soil and wet (50% sand). No-till w/ Deep shank. 3-year rotation. Problem with high alkalinity. A no-till field in soy with clear evidence of corn stover. Forested on south end and slight slope toward north end, but somewhat hilly. Some weeds, dark and sandy soil ~ 50% sand. Field is on a three-year rotation – use of deep shank tillage in some years. Tends towards high alkalinity, which has been problematic.	Dumfys - Corn, sandy, lots of rocks. Tilled. A bit of litter. A little less sandy. Some oxides. Sloped. Lots of topography (variable). Field slopes down the road steeply and then plateaus about 50 meters in. Construction equipment – clearing of trees to make field bigger – trees mostly on south (?) side – to the left coming from the road. Some low spots with drainage problems. Has been in corn – sandy soil with lots of rocks, tilled with a bit of litter left and some evidence of oxidation and poor drainage. High degree of topographical variability.
7	Wheat (good) - Good soil structure. In beans. Some moss on sand. Lots of weeds. Lots of rocks not on surface but deeper. Some good structure. Well aggregated. Some oxidation. Towards the 'back' of the land. In beans – perhaps 10-days since planting. Some moss on field and quite weedy. Quite rocky, but more in sub-surface – small glacial till. Good soil structure and well-aggregated. Some evidence of oxidation.	Alfalfa - Many earthworms. Mossy cover on soil. Dark and decent structure. Some spots more compact than others. Towards the back of the 'best' field. Lots of weeds and moss on surface. Soil is 'dark' with decent structure. Compaction for penetrometer seems highly variable. Lots of evidence of earthworms.

Table 5.5 (cont'd)

Farm Number	Choice	Non-row crop
5	Planted to wheat with a good stand. Highly compacted 53.3 ±9.1 psi at 6 inches (69±8.3 at 18 inches). Structure is poor – soil is a heavier clay. Large field planted to red wheat. Soil is much heavier – much higher clay content? Appears to be much more compacted. Crop looks good. Appears well-drained.	Sloping buffer strip. Full of Dandelions. Some water and drainage issues at lower part. Decent structure. 36 ± 7 psi at 6 inches, 74±10 at 18. Across the road 'High' field. Tons of dandelions in spite of being out of production. Some areas must be highly disturbed. Other areas have mixed grasses. Appears to have drainage problems, but soil does have dark color and decent structure.
6	Harvey's - Crumbly soil (sandier than Chris field). Corn and no litter. Some spots are clotty and muddy. 80-day corn. Coming out of wheat. Woods on right side as you enter the field. Soil is 'crumby' but sandier than field 'A'. Extensive tillage – almost no litter in evidence – more soil clods and 'muddy' with some evidence of poor drainage. Field coming out of wheat and going into 80-day corn.	Gregg's - Wheat crop looks good. Extremely compacted. Plow pan, tough dirt, very sandy and dry. No organic matter. Some corn litter. No-till. Listed as 'unmanaged' but really in wheat. 'Extremely' compacted soil – almost surely a plow pan, although based on corn litter it looks like the wheat was planted with no-till drill. Soil extremely sandy and dry with very little evidence of organic matter. Very large field – slopes toward the western edge down to woods – hard to capture variability.
7	Wheat - Soil is well-aggregated but evidence of a plow pan – highly compact especially at sub-surface. Was wheat going into hay. Pretty good organic matter. Oxides. Off to the side of the house/barn. Gently sloping land. Pretty high organic matter by visual inspection. How does he use manure from his few cows? Some evidence of oxidation and poor drainage.	Hay - Very dry. Very compact. Mixture of hay and orchard grass. Lots of diversity in insects. Variable green color in field – dark green strips/banding – either variable drainage or older drain tile in sub-surface. Highly productive for grower. Decent mix of diversity – lots of orchard grass.

Table 5.5 (cont'd)

Farm Number	Best	Worst
8	Eva's - Freshly tilled. Earthworms. Good structure. Coming out of wheat.	Lewaneowski - Surface looks cracked and dry. Some weeds. Surface residue. Crusty lots of stones. A little bit of clay deeper past 3 cm. Came out of wheat.
9	STR-#5 - Slightly less compacted. Blocky. A few earthworms.	STR-#3 - Very unsuccessful in penetrating soil. Wouldn't get below 6". Highly compact. Blocky. Small amounts of oxidation. OK moisture.
10	Home - Sandy loam, uniform corn and beans great yields clear rye residue, no earthworms. Known as 'Home' field. Typically yields are great for corn and beans. Sandy loam. Clear evidence of a rye cover crop. Little to no soil structure and no evidence of earth worm activity.	Vineyard - Hay field, sandy not good growing soil lower part does well. "not good growing soil' according to owner. 'Lower' area does better in terms of yield. Has been used for hay, but evidence of a corn crop. Also some more land being cleared. Slight rolling in field with line of woods to the south.
11	Lyle - Mostly clay; soybeans; no-till; lots of corn litter; fairly weedy; different species of weeds; thicker soil (clay texture), well structured, plow pan at 10-15"	Mortimer - Even and not many hills; some gravel, looks sandy; some corn litter; not great aggregation; lots of earthworms; some parts are dark and clayish; less litter than Lyle
12	Carmichael - Sandy loam. 31 acres of corn. Dark, more aggregates at deeper regions. Hilly. Variable in organic matter content.	Tribal - Some regions of nitrogen stress. No-till, cover crop, corn, air seeded to annual rye. Very sandy w/ earthworms. Between 7 and 8 inches deep different layer horizon top universally mixed.
13	Dad - Mostly plain not many hills, soybean crop, lots of corn residue (litter). Corn last year. Dark sand holds together. Narrow spacing between rows. No-till	Soy low - Doesn't hold together very well, no-till. Some grassy spots. Similar to pivot. Lots of corn litter. Very sandy.

Table 5.5 (cont'd)

Farm Number	Choice	Non-row crop
8	Home - Soybean field. Hard, crusty surface. Lots of residue. Darker soil/good structure. Oxidation. Good aggregation.	Buza - Been in pasture for years. Lots of rocks. Thick grass.
9	STR-#4 - More compacted within row (can't get below 6"). Doesn't go below middle of row. Slightly sandy. Structure is good. Earthworms.	Rye - Dry. Cracked at surface. Compacted. Can't penetrate below 5"!
10	Home Hay - 10 acres of hay, 4 year old, east of house; row crop; had been a pasture field. About 10 acres has been in alfalfa for 4 years. This is east of the house and field 'A'. Steep slope down to road and then level for most of the rest of the field – surrounded by woods to the north and east. Soil appears heavily compacted, especially at the subsurface. It was recently harvested – some areas not yet bailed. Evidence of earthworms and numerous grubs in areas where sampled.	Krajewski - Had cattle there wet low ground and swampy, soils black, slimy and cold great soil, good aggregation, dark soil (but sandy) pasture very vegetated, great organic matter, lots of flowers. This ground had cattle. It is low and wet. High level of plant diversity (lots of forbs in bloom) and seemingly highly productive (mostly chest high growth). Soils were black, 'slimy,' and cold – muck-like. But excellent aggregation in spite of sandiness. High level of organic matter.
11	Home -No-till beans; cows grazing; lots of corn litter; sandy, but good structure; earthworms; weedy.	Bellis (hay field) - All hay and irrigated mixture of alfalfa and some grass; great soil structure.
12	Center pivot - Clay, sand, gravel (wide range); variation in plant growth (tall short plants); corn; lots of hills; patchiness across field. Plow pan at about 12" to 15."	Vineyard/orchard - Within row less compaction, between rows more compaction.
13	Pivot - Narrow rows. Scattered cropping. Lots of litter (corn). Dark soil (sandy). Some grubs. Shaded regions on the borders. No-till	Forest - Lots of vegetation. Lots of shade. Thick brush. Dark rich soil. Organic matter. No compaction. Moist.

Table 5.6 Responses from 13 farmer interviews regarding soil testing and information used for soil management.

Question	Soil Testing and Management	# of farmers
How often do you soil sample?	Test individual fields every 2-4 years.	13
Which soil test parameters are you most interested in checking?	Fertilizer recommendation	10
	pH for lime recommendation	8
	Organic matter content	7
	Micronutrient concentration	3
	Match up nutrient contents with soil types and yield maps	2
How do soil test results inform your soil management decisions?	Target fertilizer application rates for yield goal	10
	Use (or try to use) plant tissue tests to better adjust side dress	3
	Dial fertilizer recommendations into variable rate equipment.	3
	Relate test results to number of field passes and soil compaction	2
	Avoid phosphorus excesses by adjusting crop rotation or application	2

Table 5.6 (cont'd)

Question	Soil Testing and Management	# of farmers
How do you assess or understand soil quality?	Physical characteristics (e.g. does not crust, 'works well')	9
	How water acts (e.g. drainage, ease of getting equipment into field)	3
	Organic matter content	3
	Rotation type (e.g., soil under a long rotation has better quality)	3
	Disease-free fields	2
	Healthy soil biology	1
	Chemical characteristics (e.g. pH, CEC)	1
Which sources of information do you use to inform soil management?	Word of mouth (neighbors, family)	9
	Experimentation with or inspection of own fields (or neighboring fields)	8
	Soil Testing	6
	Reading/Literature (trade magazines, the Web, books)	6
	Extension resources (including meetings or conferences)	4
	Market prices	3
	Sales representatives (fertilizer, seeds etc.)	3

Table 5.7 Responses from 13 farmer interviews regarding their selection of field types for soil sampling on their farms.

Question	Why did you select this field	# of farmers
Best	High yielding field	10
	The soil ‘works’ well	5
	Takes good care of the field	5
	Reliable/less variable to manage	4
	No apparent disease	4
	Uses field to test new ideas/experiment	2
Worst	Low yielding field	9
	Soil 'works' poorly (e.g. forms soil crust)	9
	Field not ‘reliable’	4
	Moisture problems	4
	Compaction problems	3
	History of poor management	3
	Soil chemistry is ‘off’	2
	Disease problems	1

Table 5.7 (cont'd)

Question	Why did you select this field	# of farmers
Choice	Physical problem (e.g. compaction, crusting)	7
	Lower yield, ranging from 'OK' to 'poor'	6
	History of poor management (known or assumed)	4
	Field highly variable	3
	Curious about field, no explicit problems	2
	Unsure what to do for management	2
Non-row crop	Hay field	7
	Field margin	3
	CRP/wood lot	2
	Buffer strip	1

Table 5.8. Responses from 13 farmer interviews regarding their management of fields selected for soil sampling on their farms.

Management based on field type		Best	Worst	Choice
Tillage	No till	4	5	4
	Chisel	4	5	2
	Mix (e.g. chisel, sub-soiler)	5	3	7
Crop/rotation	C-S-W	4	4	3
	C-S	5	4	5
	Other (e.g. W-S, hay)	3	4	5
	Cover crop use	5	8	5
Manure	Currently	4	2	1
	Past management	2	1	0

C=corn, S= soybean, W = Wheat

Table 5.9 Condensed responses from 13 farmer interviews regarding soil health test results from their farm.

	Soil parameter (# of farmers who commented on measure)		Summarized feedback
Soil Health metrics	Physical	Soil aggregate stability (AS) (6)	Farmer who commented on AS and AWC felt the values in the soil test corresponded to their experience across selected fields. Most had a ready agronomic explanation for why a number might be low or high for a given field. For soil compaction, however, there was much more confusion. Some felt it did not reflect their experience of a field and some were concerned and asked if they should alter their management based on comparison between field types.
		Available water capacity (AWC) (7)	
		Soil compaction (6)	
	Biological, Carbon	Total soil organic matter (OM) (9)	Most farmers were aware of total OM across different field types. In two counties with coarsest soils there was more commentary over soil organic matter/carbon. Those with 'heavier' soils were more likely to say they could not easily change their OM value. A large number wanted to better understand shorter term carbon (POXC and PMC) values, and commented directly on how they could change practices to raise it in fields with lower values to bring it closer to fields with higher faster cycling carbon pools.
		Permangenate oxidizable carbon (POXC) (2)	
		Potentially mineralizable carbon (PMC) (10)	
	Biological, Nitrogen	Potentially mineralizable nitrogen (PMN) (4)	Of the farmers that commented on PMN, all wanted to know how they could increase this value in the field to extend nitrogen release. One readily explained a high PMN value as due to recent history of including a perennial legume (alfalfa) in the particular field. Two farmers understood nitrification as a process occurring in their fields and three had used inhibitors.
		Nitrification (3)	
	Traditional field crops test	pH, phosphorus, potassium, calcium, magnesium, ammonium, nitrate (9)	In reviewing these parameters for all of their fields, farmers offered the most feedback on values for pH and phosphorus, already having an expectation for the range of these values for different fields. Some farmers also explained why a specific field had a low or high value for a given parameter, given their prior experience with testing or management of a field.

Table 5.10 Condensed responses from 13 farmer interviews regarding approach to soil management on their farms.

Themes	Summarized feedback	Quotes
Organic matter, time and place	In reflecting on testing results, nearly all growers valued organic matter and wanted to increase it, but they differed in terms of approach to management.	"It's not an overnight fix on that ground, we fixed up a lot of ground that has not been into farming over the years, and we tried to build that ground up."
	Farmers on finer soils, with generally higher organic matter values, regardless of field types, were less likely to manage for increasing organic matter, even if they wanted to. Rather, they focused on how their soil 'worked' (physically) as a metric of how to improve soil quality.	"It's so hard to raise it."
		"I am not going to live long enough to do that…"
		"I mean it's just slow, so slow going, my goodness it takes forever to change a number."
		Referring to leaving more residue on the field "...I was more impressed with the mellowness of the dirt..., I don't know that I gained a lot in yields but the dirt works a whole lot better."
	Those on coarser or more easily degraded soils place greater value on building organic matter through management.	Referring to a how he improved a field: "when we first started to farm it, it was like the desert out there, that soil would just blow away...and that goes back to the organic matter, and that takes time to build that up, that organic matter. If you can get that organic matter built up, then you can hold the water out there."
		"But if you got high organic matter, good soil CEC test, everything, it's got a good holding capacity, then your compaction isn't there. Like, say, just the general physical characteristics of the soil are so much better. "

Table 5.10 (cont'd)

Themes	Summarized feedback	Quotes
Changing crop rotation to influence measurable changes in soil health	Farmers in all counties referred to altered crop rotation (including use of cover crops) as the surest way to improve soil quality	Stated soil management goal via increased cover cropping and reduced tillage: "Keep it there, whatever I put on".
		Referring to using sorghum to improve degraded fields: ".. I think that's one of the main things...is that root system is just breaking things back up and allowing water to get through the soil…it suppresses weeds, there are no weeds growing.
		Referring to farmer practices on best fields he has seen: ",… he rotated into a rye cover … he'd always put rye in between [beans]…. he rotated oats into it he'd never grow a kernel of corn, he'd seed with clover and that sort of thing, and that's soil health, that's definitely reflected on his ground over time"
	But altering rotation was constrained, with some saying they could not do it or faced constraints.	"I'd like to get quaternary rotation if I can do it profitably…but I have a hard time making money the year corn was high. I would like the narrow rotation, no question about it."
	Reflecting on differences in soil health metrics between different field types, farmers wanted to be able to use them if they had a better sense of how to act on the numbers and if this testing would give them more control of their soil management	"…I'd like to see more stable numbers that say: 'ya I'm making progress, or no I'm not…"
		Referring to mineralizable carbon: "So it's there, it just needs to get mineralized. How do you trick it into mineralizing that? What do you put on to trick it?"
		"…If we can change that, if there's a way to change it. That's the big thing. For me, it's one thing to say, well you got a problem there. What's the implementation of the change in that and how do I change it? I want to change it."

REFERENCES

REFERENCES

Antle, J.M., Capalbo, S.M., Elliott, E.T., Hunt, H.W., Mooney, S., Paustian, K.H., 2001. Research Needs for Understanding and Predicting the Behavior of Managed Ecosystems: Lessons from the Study of Agroecosystems. Ecosystems 4, 723–735. doi:10.1007/s10021-001-0041-0

Baumgart-Getz, A., Prokopy, L.S., Floress, K., 2012. Why farmers adopt best management practice in the United States: A meta-analysis of the adoption literature. J. Environ. Manage. 96, 17–25. doi:10.1016/j.jenvman.2011.10.006

Bhardwaj, A.K., Jasrotia, P., Hamilton, S.K., Robertson, G.P., 2011. Ecological management of intensively cropped agro-ecosystems improves soil quality with sustained productivity. Agric. Ecosyst. Environ. 140, 419–429. doi:doi: 10.1016/j.agee.2011.01.005

Blesh, J., Wolf, S.A., 2014. Transitions to agroecological farming systems in the Mississippi River Basin: toward an integrated socioecological analysis. Agric. Hum. Values 31, 621–635. doi:10.1007/s10460-014-9517-3

Cherry, K.A., Shepherd, M., Withers, P.J.A., Mooney, S.J., 2008. Assessing the effectiveness of actions to mitigate nutrient loss from agriculture: A review of methods. Sci. Total Environ. 406, 1–23. doi:doi: DOI: 10.1016/j.scitotenv.2008.07.015

Chouinard, H.H., Paterson, T., Wandschneider, P.R., Ohler, A.M., 2008. Will farmers trade profits for stewardship? Heterogeneous motivations for farm practice selection. Land Econ. 84, 66–82.

Diaz, R.J., Rosenberg, R., 2008. Spreading Dead Zones and Consequences for Marine Ecosystems. Science 321, 926–929.

Doane, T.A., Horwáth, W.R., 2003. Spectrophotometric Determination of Nitrate with a Single Reagent. Anal. Lett. 36, 2713–2722. doi:10.1081/AL-120024647

Dobbs, T.L., Pretty, J.N., 2004. Agri-Environmental Stewardship Schemes and "Multifunctionality." Appl. Econ. Perspect. Policy 26, 220–237. doi:10.1111/j.1467-9353.2004.00172.x

Drinkwater, L., Snapp, S., 2007. Understanding and managing the rhizosphere in agroecosystems. Elsevier: Burlington, MA, USA.

Drinkwater, L.E., Cambardella, C.A., Reeder, J.D., Rice, C.W., 1996. Potentially mineralizable nitrogen as an indicator of biologically active soil nitrogen, in: Methods for Assessing Soil Quality. Soil Science Society of America, Madison, WI, pp. 217–229.

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 Sci. Soc. Am. J. 64, 613–623.

Havlin, J.L., Beaton, J.D., Tisdale, S.L., Nelson, W.L., 2005. Soil fertility and fertilizers: An introduction to nutrient management. Pearson Prentice Hall Upper Saddle River^ eNJ NJ.

Karlen, D.L., Andrews, S.S., Wienhold, B.J., Zobeck, T.M., 2008. Soil Quality Assessment: Past, Present and Future.

Kettler, T.A., Doran, J.W., Gilbert, T.L., 2001. Simplified Method for Soil Particle-Size Determination to Accompany Soil-Quality Analyses. Soil Sci. Soc. Am. J. 65, 849. doi:10.2136/sssaj2001.653849x

Lambert, D., Sullivan, P., Claassen, R., Foreman, L., 2006. Conservation-Compatible Practices and Programs: Who Participates? (Economic Research Report No. 14). USDA Economic Research Service.

Michalak, A.M., Anderson, E.J., Beletsky, D., Boland, S., Bosch, N.S., Bridgeman, T.B., Chaffin, J.D., Cho, K., Confesor, R., Daloğlu, I., DePinto, J.V., Evans, M.A., Fahnenstiel, G.L., He, L., Ho, J.C., Jenkins, L., Johengen, T.H., Kuo, K.C., LaPorte, E., Liu, X., McWilliams, M.R., Moore, M.R., Posselt, D.J., Richards, R.P., Scavia, D., Steiner, A.L., Verhamme, E., Wright, D.M., Zagorski, M.A., 2013. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. Proc. Natl. Acad. Sci. doi:10.1073/pnas.1216006110

Moebius, B.N., van Es, H.M., Schindelbeck, R.R., Idowu, O.J., Clune, D.J., Thies, J.E., 2007. Evaluation of laboratory-measured soil properties as indicators of soil physical quality. Soil Sci. 172, 895–912.

Moebius-Clune, B.N., Moebius-Clune, D.J., Gugino, B.K., Idowu, O.J., Schindelbeck, R.R., Ristow, A.J., van Es, H.M., Thies, J.E., Shayler, H.A., McBride, M.B., Wolfe, D.W., Abawi, G.S., 2016. Comprehensive Assessment of Soil Health – The Cornell Framework Manual, Edition 3.0. ed. Cornell University, Geneva, NY.

Moebius-Clune, B.N., van Es, H.M., Idowu, O.J., Schindelbeck, R.R., Moebius-Clune, D.J., Wolfe, D.W., Abawi, G.S., Thies, J.E., Gugino, B.K., Lucey, R., 2008. Long-Term Effects of Harvesting Maize Stover and Tillage on Soil Quality. Soil Sci. Soc. Am. J. 72, 960. doi:10.2136/sssaj2007.0248

Norton, J.M., Stark, J.M., 2011. Regulation and measurement of nitrification in terrestrial systems. Methods Enzym. 486, 343–368.

Obade, V. de P., Lal, R., 2016. A standardized soil quality index for diverse field conditions. Sci. Total Environ. 541, 424–434. doi:10.1016/j.scitotenv.2015.09.096

R Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reynolds, W.D., Topp, G.C., 2008. Soil water desorption and imbibition: tension and pressure techniques. Soil Sampl. Methods Anal. 2nd Ed. Can. Soc. Soil Sci. Taylor Francis LLC Boca Raton FL 981–997.

Robertson, G.P., Gross, K.L., Hamilton, S.K., Landis, D.A., Schmidt, T.M., Snapp, S.S., Swinton, S.M., 2014. Farming for Ecosystem Services: An Ecological Approach to Production Agriculture. BioScience 64, 404–415. doi:10.1093/biosci/biu037

Robertson, G.P., Wedin, D., Groffman, P.M., Blair, J.M., Holland, E.A., Nadelhoffer, K.J., Harris, D., 1999. Soil carbon and nitrogen mineralization, nitrification, and soil respiration potentials, in: Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York, pp. 258–71.

Romig, D.E., Garlynd, M.J., Harris, R.F., McSweeney, K., 1995. How farmers assess soil health and quality. J. Soil Water Conserv. 50, 229–236.

Schipanski, M.E., MacDonald, G.K., Rosenzweig, S., Chappell, M.J., Bennett, E.M., Kerr, R.B., Blesh, J., Crews, T., Drinkwater, L., Lundgren, J.G., others, 2016. Realizing Resilient Food Systems. BioScience biw052.

Shennan, C., 2008. Biotic interactions, ecological knowledge and agriculture. Philos. Trans. R. Soc. Lond. B Biol. Sci. 363, 717–739. doi:10.1098/rstb.2007.2180

Singer, J.W., Nusser, S.M., Alf, C.J., 2007. Are cover crops being used in the US corn belt? J. Soil Water Conserv. 62, 353–358.

Sinsabaugh, R.., Reynolds, H., Long, T.., 2000. Rapid assay for amidohydrolase (urease) activity in environmental samples. Soil Biol. Biochem. 32, 2095–2097. doi:10.1016/S0038-0717(00)00102-4

Stuart, D., Gillon, S., 2013. Scaling up to address new challenges to conservation on US farmland. Land Use Policy, Themed Issue 1-Guest Editor Romy GreinerThemed Issue 2- Guest Editor Davide Viaggi 31, 223–236. doi:10.1016/j.landusepol.2012.07.003

Walter, G., Wander, M., Bollero, G., 1997. A farmer-centered approach to developing information for soil resource management: The Illinois Soil Quality Initiative. Am. J. Altern. Agric. 12, 64–72. doi:10.1017/S0889189300007268

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating active carbon for soil quality assessment: A simplified method for laboratory and field use. Am. J. Altern. Agric. 18, 3–17. doi:10.1079/AJAA200228

Wolf, S.A., Nowak, P.J., 1995. Development of information intensive agrichemical management services in wisconsin. Environ. Manage. 19, 371. doi:10.1007/BF02471979