

ORG Project Details

Award Year 2019

11 Research Projects

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Intensive Annual Vs. Perennial Forage Cropping Strategies to Build Soil Health and Nitrogen Efficiency in Transitioning Tomato Systems

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NON-TECHNICAL SUMMARY

Keeping weed pressure low and building up soil organic matter and fertility levels are two of the most important objectives for growers transitioning ground from conventional to organic management. While little information has been available to growers in the past, recommendations for the transition period have traditionally emphasized high organic matter inputs from high-biomass crops and/or application of organic materials like composts or manures. Alternatively, some experienced growers recommend using perennial crops like alfalfa during the transition period. Uncertainty remains regarding the relative efficacy and profitability of annual versus perennial approaches to transition, especially in California tomato production systems. Processing tomatoes are an important crop in northern California, a region that produces over 95% of the tomatoes that are used for ketchup, sauces, salsa, and canning in the nation. Demand for organic processing tomatoes has been growing steadily throughout the last decade by one to two percent per year, and decreasing revenues in the conventional tomato industry could drive more producers to consider transitioning their tomato production operations to organic. Despite the steady growth in organic processing tomato production in California, little information tailored to the processing tomato industry has been made available to inform management practices and decisions during the transition to organic period. Particularly, northern California growers have expressed a need for information on cropping strategies and rotation options, including cover crops, annual crops, and perennial forages, as well as a more detailed understanding of soil organic matter, nutrient, and soil microbial community trends as the transition-to-organic period progresses. This study aims to provide California tomato growers with information they have requested on the i) time scale of conversion of soil fertility and structure during the transition to organic practices, ii) the optimal rotational crops for N release and soil carbon building in Mediterranean cropping systems, and iii) comprehensive financial analyses of incremental costs and benefits to maximize net revenues (or minimize costs) for growers during transition and subsequent certified organic production. These results will be disseminated through innovative educational and extension methods, including hands-on soil health and winter cover crop management workshops and audio programming for radio and podcast dissemination.

OBJECTIVES

Monitor and analyze soil health indicators (including biological, chemical, and physical soil measurements) and nutrient release changes in intensive annual and perennial forage cropping systems during the transition period, and evaluate effects on subsequent certified organic tomato productivity. Evaluate the effect of winter livestock grazing during the transition period on root growth, soil health indicators, nutrient release, and evaluate effects on subsequent certified organic tomato productivity. Compare weed biomass, weed community composition, and the weed seedbank during the transition period, and weed populations in the first year of certified tomato production. Construct Land Use System models as frameworks for multi-year economic analyses of financial costs and benefits for the intensive annual and perennial forage transition systems for certified organic tomato production, compared to conventional control systems for conventional processing tomato production. Disseminate project findings through field walks, demonstration plots, and soil health workshops, as well as novel outreach methods including the development of audio communications materials (podcasts and radio programs).

APPROACH

• Experimental design and cropping systems: The intensive annual and perennial forage transition systems will be established in a randomized complete split-plot design with cropping system as the main plot treatment, and grazing as the split-plot treatment, in four replications in 9 x 60m plots on land undergoing transition to certified organic production at the RRSFAF. The conventional control system will be established on adjacent non-certified land in a randomized complete block design with the same soil type as the certified organic ground. Conventional control systems will be measured for productivity (yield and harvest quality) parameters, primarily serving as conventional controls for the economic analysis. The conventional controls will consist of a conventional mimic of the intensive annual system (a sudangrass-corn silage-garbanzo rotation with conventional mineral fertilizer inputs), to enable analysis of the effects of organic management and inputs (poultry manure compost, winter cover crops, and grazer incorporation) on productivity and economic outcomes. A nearby conventional stand of alfalfa will be similarly used as a conventional control for the transition perennial forage system. • Experimental approach: Soil microbial biomass and activity will be measured at three time points per year: prior to planting during active growth of the cash crop, and during active growth of the winter cover crop (approximately April, June and March, respectively) in the annual intensive system, and these sampling times will be mimicked in the perennial transition and conventional control systems. Sampling will be performed in split plots of the transition systems to evaluate the impact of winter grazing. Soil cores will be collected to a total depth of 30 cm, 6 cores per plot, and aggregated into one sample per plot. The microbial communities will be characterized using PLFA analysis. Soil pH, bulk density, soil available P and K, total N, total carbon, and aggregate stability (a metric of soil structure) will be measured prior to the initiation of the organic transition and conventional control systems, and after the transition period concludes, just prior to planting the first certified organic crop. Aggregate stability will be measured and analyzed according to the methods of Elliott (1986). Weed biomass will be measured in early March prior to termination of winter cover crops in the grazed and non-grazed split plots, and at peak biomass production of summer crops in the organic intensive annual system. The viable or germinable weed seedbank composition will be measured at the beginning and end of the transition period in all organic split plot treatments and in the main plots of the conventional systems, similar to soil microbial community composition, using the greenhouse germination technique described by Ball and Miller (1989). Using a Land Use System (LUS) economic model (Tomich et al. 2005; Vosti et al. 2005), we will quantify and evaluate tradeoffs associated with different organic transition system strategies (intensive annual vs. perennial forage), to calculate net present values (NPV) at a range of relevant discount rates and internal rates of return (IRR) for each system spanning the full transition period. Net Present Value estimates will be compared among organic transition systems and their conventional control systems to compare financial tradeoffs growers face arising from transition strategies and certified organic production. LUS models will be completed for the transition period in year 3 and will be updated to include the discounted net revenues of certified organic crop phase over a typical rotation beginning at year 4 to incorporate the economic effects of transition system ongoing effects on subsequent crop productivity and net financial returns to growers. Progress 09/01/19 to 08/31/22 Outputs Target Audience: We presented our results to two main audience groups: Farmers and other field practitioners, and soil health scientists. The farmers we interacted with were mostly operation managers of field crops in Yolo County, CA who attend field meetings through University of California Cooperative Extension. We interacted with soil health scientists by inviting them to our lab meetings, or through attendance in scientific meetings. Changes/Problems: We had no choice but to change our experimental design to eliminate the conventional treatment after the first year and focus the remainder of our field study on comparing the two organic transition treatments. Costs for field operations at UCD have increased substantially beyond initial estimates and there was not sufficient budget to maintain the conventional treatment. In order to ensure some results are obtained from organic production within the timeframe of our funded project, we terminated organic transition plots 5 months ahead of schedule and planted them into organic corn in June 2022. We used corn as a substitute crop for tomatoes since it also has high nitrogen requirements and was within budget. The corn plots were used to estimate responses to the different

treatments in: crop yield (biomass), weed seedbank, perennial weed pressure, and soil health parameters. What opportunities for training and professional development has the project provided? We have had a total of 6 undergraduate students work on this project throughout its duration, allowing them training into fieldwork, lab work, and some basic scientific analyses. The tasks covered by the undergrad trainees included sampling and recording crop and weed biomass, measuring infiltration and penetration resistance in the field, measuring soil aggregate stability, soil nitrogen and soil carbon in the lab, and assisting in soil incubation experiments. We trained four student assistants, one junior assistant and one postdoc on a protocol for multiple simultaneous field infiltration measurements, and used the method in this project and others, exploring soil health and function outcomes of various soil amendments and soil conservation techniques. In our collaboration with Amelie Gaudin's lab on Integrated Crop Livestock Systems, we trained one PhD student on field and lab methods in weed science, including cover measurements, weed identification, and seed germination assays. Results from this project will be integrated into that student's thesis and a possible publication. How have the results been disseminated to communities of interest? We used extension events to present some of our findings to growers and other industry stakeholders in the last two years. We presented some of our preliminary findings in an online event in January 2021, and towards the end of the project we presented results at a farm practitioners field day (organized by UCCE Delta Crops Advisor), and at an online lecture in an international academic institute (Arava Institute for Environmental Studies seminar). We will present many of our findings in the Alfalfa Growers Meeting, to be held in November 2022 at San Diego. List of presentations: Management Options for Transition to Organic, online field day on: alternative management in organic tomatoes, January 5, 2021, 33 participants Weed biomass and forage yield responses to integration of short-term grazing in organic fields, poster, March 18, 2022, UC Davis Postdoctoral Research Symposium, 124 participants Alternative management of organic field crops in California: sustainability through diversity, May 23, 2022, AIES online seminar, 12 participants Soil Health Outcomes of Different Rotation Strategies in Transition to Organic, July 28, 2022, UCCE Compost field day, 15 participants Soil Health and Biological Benefits of Alfalfa Rotations, November 14-17, 2022, World Alfalfa Congress, 200 participants What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported Impacts What was accomplished under these goals? 1. Monitor and analyze soil health indicators and nutrient release changes Nutrient levels were similar among all plots during plot setup and throughout the first two years. Nitrogen dynamics are different between rotations, but other nutrients and SOM have not shown significant differences. Following forage termination and organic corn planting in the third year, alfalfa plots have significantly higher residual nitrate levels. Physical soil health parameters have been estimated for baseline status. Infiltration tests revealed alfalfa plots have a much higher saturated hydraulic conductivity than the annual rotation, although a larger sample size is required for robust conclusions to be made. Aggregate stability of near-surface soil has been similar throughout the entire transition period. Soil incubation experiments showed in spring 2021, annual rotation soils showed higher levels of potentially mineralizable carbon (PMC), while PMN was similar in both rotations. Potential substrate limitation to mineralization rates was explored by measuring soil and root total carbon and nitrogen (TC and TN), revealing 10% lower TC and TN in alfalfa, likely due to lower compost inputs. The higher PMC corresponded with higher TC in annual rotation soils. One year later, following termination and before planting organic corn, both rotations had similar PMC and TC levels, while alfalfa soil had higher PMN, corresponding with higher soil nitrogen. PLFA revealed only small differences among the different treatments in terms of total biomass and biomass ratios among different groups. Microbial biomass carbon (MBC) was likewise similar among rotation treatments. 2. Evaluate the effect of winter livestock grazing during the transition period on root growth, soil health indicators, and nutrient release. We found no significant effects of crop or grazing treatments on soil nutrient availability and organic matter content in year 1 nor consistent effects of grazing on any parameter, including TN and TC, aggregate stability or crop yields, during and following transition. Grazing caused a slight reduction (~10%) in some values of soil health testing, including TN, TC, and mineralizable carbon in the second spring, but no differences were found in the third spring. It is important to note that sheep did not graze the field in the third winter, since the drought led to very low biomass availability. Overall, we conclude that the short-term grazing we utilized had very little impact on any soil parameters. Similar results were found in a parallel field experiment on organic tomato plots, further supporting our conclusion that sheep grazing will not degrade soil health or functions. Root biomass was slightly higher during spring in the annual rotation compared to Alfalfa. As Alfalfa has slower growth rate in winter, that outcome was expected for the establishment year. Second year alfalfa root biomass was observed to be 10-fold higher than first-year alfalfa. Our results point to alfalfa having a higher potential root biomass, but also higher spatial variability, compared to annual forages planted at higher densities. The alfalfa rooting system is typically deeper than annual forages, but we did not test deeper soil layers. 3. Compare weed biomass, weed community composition, and the weed seedbank during the transition period. Whole community and species-specific seedling densities were unrelated to late season weed biomass, with most biomass being produced by two species with sparse seedling densities (Mustard and lambs quarters in winter, tumble pigweed in summer). Weed cover and abundance, measured in quadrates in summer 2021, was unaffected by grazing. One exception seems to be a slight reduction in abundance of the weed Tumble pigweed in grazed plots. This contrasts to data

collected in another grazing study, showing increased broadleaf weed biomass, mostly pigweeds, in grazed plots. Weed seedbank, as tested by a field survey of sprouting weeds and germination in controlled greenhouse assays, showed no difference among any treatment combinations. Sheep grazing effects on winter and spring weed cover could not be assessed thoroughly, as the WCC and the second-year alfalfa effectively controlled weeds. Summer weed cover, although significantly limited by annual forage crops, did not differ among the grazing treatments. Preliminary observations from a parallel shows potential for spring grazing to reduce weed pressure during organic tomato cultivation. A field survey revealed lower abundance but similar biomass of Barnyard grass, a summer annual weed common in organic fields. Further studies showed the grazed soil had much lower density of Barnyard grass seeds, but similar densities of the less-abundant broadleaf weeds. Altogether, our results suggest winter grazing has limited potential to control weeds in summer crop fields, and this potential is species-specific. We found no differences in perennial weed cover among the grazing treatments, alluding to the ineffectiveness of grazing only above-ground biomass of species with belowground clonal growth. Weed cover and biomass significantly reduced organic alfalfa yield in the first year (up to 40% below conventional yield), with some slight residual effect showing in second-year harvests as well. This highlights one of the main potential drawbacks of using alfalfa for the transition period, although careful management could limit weed biomass by performing an earlier first cut or by introducing grazing during the establishment year. Following the transition to organic corn, we measured annual weed seedbank and perennial weed cover in all plots. We found no differences in seedbank properties (density, composition), but did see higher cover of perennial weeds, particularly Yellow nutsedge, following alfalfa in rotation. This is another disadvantage for using alfalfa in rotation, but it can be mitigated by a deeper soil inversion prior to planting, which we did not perform.

4. We found that the method we intended to use to evaluate economic performance, the Land Use Systems model, was not the best approach for comparing a three-year period, as it would require too many assumptions of long-term trends. In place of that we prepared a partial-budget analysis, comparing only expenses and income that would differ among the rotation systems. This method is appropriate due to its focus on shorter-term economics, and due to its use in cost studies by extension specialists, making the structure and reasoning more familiar to farmers. Our base model of partial budgets, using our yield data to assess expected income and our actual inputs to assess costs, resulted in slightly better economic outcome of the annual system (ca. \$50/Ha of NPV after 3 years), mostly due to higher biomass yields (assuming the biomass is sold for forage and). As that difference is equal to relatively small (~1T/Ha) changes in biomass yield, it represents near parity in economic performance, without accounting for any differences in the first-year post-transition. Adding the yield boost we found following alfalfa to the calculation, it's likely that alfalfa would outperform the annual rotation. Additionally, we explored different scenarios of drought effects on water availability. We used a-priori assumptions regarding amount and cost of available water, and the effect of irrigation amounts on forage yield. Preliminary data indicates that alfalfa involves lower financial risk from drought years, as a field can produce >60% of the seasonal yield using ~50% of its regular water consumption. This conclusion is based on our field results and previous studies, showing that up to 70% of alfalfa seasonal yield is collected in the few cuts.

5. Dissemination--see below. Publications Progress 09/01/20 to 08/31/21 Outputs Target Audience: We presented our work, along with other related research, in January of this year. The audience, about 40 people, included: growers in organic production and considering a transition to organic tomato industry researchers extension specialists in field crops and organic production Changes/Problems: We have changed our experimental design to eliminate the conventional treatment after the first year and focus the remainder of our field study on comparing the two organic transition treatments. Costs for field operations at UCD have increased substantially beyond initial estimates and we don't have sufficient budget to maintain the conventional treatment. It will not pose a problem for our study because we can collect the data we need about the conventional treatment from annually published county-wide yields. These published data, will provide the base data we need for our economic analysis (Land Use Analysis--LUS) of the different organic transition treatments. Therefore this change will not prevent achievement of the goals of our study. What opportunities for training and professional development has the project provided? We have had several undergraduate students work on this project, allowing them training into fieldwork, lab work, and some basic scientific analyses. How have the results been disseminated to communities of interest? Results from the first year were presented in a webinar dedicated to organic tomato production, as previously reported. What do you plan to do during the next reporting period to accomplish the goals? Continued sampling and data analysis of soil and crop parameters taken in the summer of the second year allows us to see trends in system responses to our management factors. As our understanding of these trends grows, we will prepare materials to publish within scientific and other communities. We will also test hydrologic and physical soil attributes, to see how their agronomic function is affected by the different treatments. Following our initial results from soil microbial activity testing, we are re-focusing some of our efforts on exploring the role of rotation in affecting soil nitrogen and carbon cycling potential, a critical aspect in organic agriculture. This will include controlled studies using incubations, as well as enzymatic and possibly some genetic analyses. We will explore how these system attributes respond to diversity in rotation and to an annual legume crop, which will be planted in the third year of transition. Impacts What was accomplished under these goals? One of the most

striking results from our first year of research is that weed pressure is a potential limitation in integrating alfalfa in the transition period to organic management. In contrast, growing annual cover crops in the winter offers effective weed control. Whether there is potential for weed control in alfalfa using grazing has not been fully tested, but our results suggest the grazers removed relatively more weeds than alfalfa plants. Soil health changes associated with different management systems have been inconsistent, and we believe require more time to reflect differences. So far, nutrient levels are similar among treatments in the organic management systems. Summer available nitrogen levels are similar among systems despite fertilization in the conventional annual system, thus showing the potential of the organic WCC mix to supply some nitrogen for subsequent summer crops. It is important to note that these findings are early ones and our understanding and conclusions may change throughout the duration of the project, as new data is generated and analyzed. Concurrently, we have initiated an experiment on organic fertilizers in tomatoes in our long-term experimental facility. By comparing measurements of soil nutrient cycling functions in both experiments, we hope to gain a better understanding of soil fertility and plant nutrition management in organic tomato production. Progress on specific goals:

1. Monitor and analyze soil health indicators (including biological, chemical, and physical soil measurements) and nutrient release changes
 - 1.1. Nutrient levels were similar among all plots during plot setup and throughout the first year. Nitrogen dynamics are different between rotations, but other nutrients and SOM have not shown significant differences.
 - 1.2. Physical soil health parameters have been estimated for baseline status. Infiltration tests revealed alfalfa plots have a much higher saturated hydraulic conductivity than the annual rotation, although repetition with a larger sample size is required for robust conclusions to be made. Aggregate stability of near-surface soil has so far been similar; another test is planned using second-year soil.
 - 1.3. Soil incubation experiments revealed a difference in potentially mineralizable carbon (PMC-24h) between rotations, with Alfalfa soil mineralizing 25% less carbon. However, nitrogen mineralization was not different between any treatments. Additionally, nitrogen mineralization was not correlated to carbon mineralization in the alfalfa soil, but significantly so in the cover crop soil. Potential substrate limitations to mineralization rates was explored by measuring soil and root total carbon and nitrogen (TC and TN), revealing 10% lower TC and TN in alfalfa, likely due to lower compost inputs. Soils from plots that had been grazed resulted in slightly lower rates of carbon mineralization in both rotation treatments, though differences were smaller than the observed rotation effect. Another round of incubation experiments is planned to further explore these results and increase our confidence in the emerging patterns.
 - 1.4. Biological parameter tests (Microbial Carbon, PLFA, Enzyme activity) are in preparation for soils from the first and second years. Equipment failures are delaying some analyses.
2. Evaluate the effect of winter livestock grazing during the transition period on root growth, soil health indicators, and nutrient release.
 - 2.1. Throughout the first winter and summer, we found no significant effects of crop or grazing treatments on soil nutrient availability and organic matter content. Second year grazing included the alfalfa plots. Analysis of soil fertility is ongoing, and crop yields are currently being calculated.
 - 2.2. Grazing caused a slight reduction (~10%) in certain soil health parameters, including total nitrogen, total carbon, and potentially mineralizable carbon.
 - 2.3. Root biomass was slightly higher during spring in the annual rotation compared to Alfalfa. As Alfalfa has a slower growth rate in winter, that outcome was expected. Second year alfalfa root biomass was up to 10-fold higher than first-year alfalfa. Root biomass of second year alfalfa and summer cover crops is currently being analyzed for biomass and carbon and nitrogen content.
3. Compare weed biomass, weed community composition, and the weed seedbank during the transition period.
 - 3.1. Data on weed seedling densities and community composition has been analyzed. Data on weed cover and biomass has been recorded and analyzed for the spring and most of the summer season. Whole community and species-specific seedling densities were unrelated to late-season weed biomass, with most biomass being produced by two species with sparse seedling densities (Mustard and Lambsquarters). Weed cover and abundance, measured in quadrates in summer 2021, was unaffected by grazing. One exception seems to be a reduction in abundance of the weed Tumble pigweed in grazed plots. This result stands contrary to data collected in another grazing study, showing increased broadleaf weed biomass, mostly pigweeds, in grazed plots.
 - 3.2. The weed seedbank, as tested by a field survey of sprouting weeds and germination in controlled greenhouse assays, showed no difference among any treatment combinations.
 - 3.3. The impact of sheep grazing on winter and spring weed cover could not be isolated or assessed thoroughly, as the WCC and the second-year alfalfa effectively controlled weeds. Summer weed cover, although significantly limited by forage crops, did not differ among treatments. Summer weed community composition is currently under analysis. Preliminary observations from a parallel experiment (run by Gaudin's lab) shows potential for spring grazing to reduce weed pressure during organic tomato cultivation. A survey in that field revealed lower abundance but similar biomass of Barnyardgrass, a summer annual weed common in organic fields. We are running a germination assay, using an improved protocol, to assess the effect of grazing on germination and early growth of Barnyardgrass.
 - 3.4. Weed cover and biomass significantly reduced organic alfalfa yield in the first year (up to 40% below conventional yield), with some slight residual effect showing in second-year harvests as well. This highlights one potential drawback of using alfalfa for the transition period. However, weeds could be better managed by introducing an earlier first cut of the alfalfa or by including grazing during the establishment year.
4. The land use systems (LUS) model is being constructed by collecting information on expected costs and

revenue associated with application of different management scenarios. As is the case for many researchers in the past 2 years, the COVID-19 pandemic impacted our research in several ways, including the inability to hold planned outreach activities due to the cancellation of in-person programming. We do have plans to present organic alfalfa system results at an outreach event in November 2021, in collaboration with UC Davis Extension forage researchers. Additionally, we held a webinar last winter for California organic tomato growers and other practitioners ("Alternative management in Organic Tomato Production", January 5, 2021), to disseminate project results. We have so far been communicating with other researchers conducting studies on organic agriculture and alfalfa management, working to make our data useful for a wide audience. These include members of Prof. Amelie Gaudin's lab in UC Davis, who are conducting a parallel experiment with sheep grazing in established organic tomato fields. Their results will shed light on our expected outcomes with regard to the future tomato production in our plots, while our results combined with theirs will provide a larger data base and more case studies for those interested in the transition to organic production Publications

PROGRESS

2019/09 TO 2020/08 Target Audience: Nothing Reported Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? Our postdoctoral fellow has received training in advanced methods of soil enzyme and nutrient release measurements by the lab of Dr. Daniel Geisseler, and is implementing them in our project. How have the results been disseminated to communities of interest? Nothing Reported What do you plan to do during the next reporting period to accomplish the goals? Our postdoc will audit a graduate course providing an overview of the Land Use Systems model this winter quarter. We plan to have initial results on economic outcomes of the first year during the coming winter.

2020/09 TO 2021/08 Target Audience: We presented our work, along with other related research, in January of this year. The audience, about 40 people, included: growers in organic production and considering a transition to organic tomato industry researchers extension specialists in field crops and organic production Changes/Problems: We have changed our experimental design to eliminate the conventional treatment after the first year and focus the remainder of our field study on comparing the two organic transition treatments. Costs for field operations at UCD have increased substantially beyond initial estimates and we don't have sufficient budget to maintain the conventional treatment. It will not pose a problem for our study because we can collect the data we need about the conventional treatment from annually published county-wide yields. These published data, will provide the base data we need for our economic analysis (Land Use Analysis--LUS) of the different organic transition treatments. Therefore this change will not prevent achievement of the goals of our study. What opportunities for training and professional development has the project provided? We have had several undergraduate students work on this project, allowing them training into fieldwork, lab work, and some basic scientific analyses. How have the results been disseminated to communities of interest? Results from the first year were presented in a webinar dedicated to organic tomato production, as previously reported. What do you plan to do during the next reporting period to accomplish the goals? Continued sampling and data analysis of soil and crop parameters taken in the summer of the second year allows us to see trends in system responses to our management factors. As our understanding of these trends grows, we will prepare materials to publish within scientific and other communities. We will also test hydrologic and physical soil attributes, to see how their agronomic function is affected by the different treatments. Following our initial results from soil microbial activity testing, we are re-focusing some of our efforts on exploring the role of rotation in affecting soil nitrogen and carbon cycling potential, a critical aspect in organic agriculture. This will include controlled studies using incubations, as well as enzymatic and possibly some genetic analyses. We will explore how these system attributes respond to diversity in rotation and to an annual legume crop, which will be planted in the third year of transition.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? As is the case for many researchers this year, the COVID-19 pandemic has impacted our research in several ways, including the inability to hold planned outreach activities due to the cancellation of in-person programming. For example, we had planned to present project results at the annual UC Davis Alfalfa & Forage Field Day in May 2020; however, COVID-19-induced restrictions led to cancellation of in-person outreach or demonstrations to grower stakeholders. We do have plans to present organic alfalfa system results at an outreach event in May, in collaboration with UC Davis Extension forage researchers. Additionally, we will hold a webinar in the fall for western organic tomato and vegetable

producers, to disseminate project results. However, despite being still in the initial year of our three-year experimental timeline, useful information has already been provided on differences between cropping strategies during transition to organic for the challenging first year of transition. It is important to note that our understanding and our conclusions may change throughout the duration of the project, as new data is generated and analyzed. However, results from the first winter already indicate that weed pressure can be a problematic aspect for growing alfalfa in the transition period, whereas annual cover crops offer effective weed control. And, while livestock grazing has anecdotally shown potential for weed control in our plots, results for grazing in Alfalfa will only be available in seasons 2 and 3. Conversely, soil health changes have been inconsistent, and we believe require more time to reflect differences among management systems. So far, nutrient levels are similar among rotation treatments in the organic management systems. Nitrate availability shows a large contrast between organic and conventional plots following WCC termination, being higher in organic management, regardless of rotation. Summer available nitrogen levels are similar among systems despite fertilization in the conventional annual system, thus showing the potential of the organic WCC mix to supply some nitrogen for subsequent summer crops. As an addition to this organic tomato project, we have received additional funds (\$20,000) from the Organic Farming Research Foundation to study in-season fertigation of OMRI-approved products to enhance N uptake and fertility in certified organic tomatoes. We have established relationships with organic fertilizer companies and researchers to form partnerships to improve N management in processing tomato rotations. We have so far been communicating with other researchers in organic agriculture and alfalfa management, working to make our data useful for a wide audience. These include Amelie Gaudin's lab in UC Davis, who are conducting a parallel experiment with sheep grazing in established organic tomato fields. Their results will shed light on our expected outcomes with regard to the future tomato production in our plots, while our results will combine with theirs to increase the utility of both projects as applicable research. Due to the initial stage of the project, any publication or extension based on this project requires more time for more conclusive and consistent results. Concurrently, we have initiated an experiment on organic fertilizers in tomatoes in our long-term experimental facility. By comparing measurements of soil nutrient cycling functions in both experiments, we hope to gain a better understanding of soil fertility and plant nutrition management in organic tomato production.

Progress on specific goals: 1. Monitor and analyze soil health indicators (including biological, chemical, and physical soil measurements) and nutrient release changes chemical health indicators have been analyzed for three time-points during the first year of the experiment. Nutrient levels were similar among all plots during plot setup. Nitrogen dynamics are different between cropping systems, other nutrients and SOM have not shown significant differences. Physical soil health parameters have been estimated for baseline status. Nutrient release dynamics (Nitrogen mineralization) has been tested, and analysis is ongoing. Biological parameter tests (Microbial Carbon, PLFA, Enzyme activity) are in preparation for soils from the first year. Evaluate the effect of winter livestock grazing during the transition period on root growth, soil health indicators, and nutrient release Data for root growth and soil health parameters has been recorded for evaluation of livestock effects, and analysis of first year data is undergoing. Throughout the first winter and summer, we found no significant effects of crop or grazing treatments on soil nutrient availability and organic matter content. Root biomass was slightly and non-significantly higher during spring in the annual rotation compared to Alfalfa. As Alfalfa has slower growth rate in winter, that outcome was expected. Compare weed biomass, weed community composition, and the weed seedbank during the transition period. Data on weed seedling densities and community composition has been analyzed. Data on weed cover and biomass has been recorded and analyzed for the spring season and most of summer season. Whole community and species-specific seedling densities were unrelated to late-season weed biomass, with most biomass being produced by two species with sparse seedling densities (Mustard and Lambsquarters). In Alfalfa, plot-scale weed cover, measured using point transects, was positively correlated with sample-scale weed biomass. Sample-scale weed biomass exerted a strong negative effect on Alfalfa biomass, reducing first-cut yields by 50-75% in organic plots compared to conventional plots. High levels of weed biomass also reduce the hay quality of Alfalfa, resulting in lower expected revenue. Later-season organic Alfalfa yields showed an improvement in terms of weed biomass yet were highly variable, due to loss of Alfalfa cover, and averaged 70-85% of conventional Alfalfa yields. Sheep grazing effects on winter and spring weed cover could not be assessed, as the WCC effectively controlled weeds, and Alfalfa plots will only be grazed in years 2 and 3. Preliminary observations from a parallel experiment (run by Gaudin's lab) shows potential for spring grazing to reduce weed pressure during organic tomato cultivation. Construct Land Use System models as frameworks for multi-year economic analyses Data for Land Use Systems model is being collected and assessed. This includes field operations expenses and input amounts and costs, as well as yield and feed quality of winter cover crops and Alfalfa. Alfalfa hay is of high quality in both conventional and organic systems, with organic hay showing improvement in quality, as measured in Relative Feed Value (RFV) throughout the season. **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 No publications reported this period.

2020/09 TO 2021/08 What was accomplished under these goals? One of the most striking results from our first year of research is that weed pressure is a potential limitation in integrating alfalfa in the transition period to

organic management. In contrast, growing annual cover crops in the winter offers effective weed control. Whether there is potential for weed control in alfalfa using grazing has not been fully tested, but our results suggest the grazers removed relatively more weeds than alfalfa plants. Soil health changes associated with different management systems have been inconsistent, and we believe require more time to reflect differences. So far, nutrient levels are similar among treatments in the organic management systems. Summer available nitrogen levels are similar among systems despite fertilization in the conventional annual system, thus showing the potential of the organic WCC mix to supply some nitrogen for subsequent summer crops. It is important to note that these findings are early ones and our understanding and conclusions may change throughout the duration of the project, as new data is generated and analyzed. Concurrently, we have initiated an experiment on organic fertilizers in tomatoes in our long-term experimental facility. By comparing measurements of soil nutrient cycling functions in both experiments, we hope to gain a better understanding of soil fertility and plant nutrition management in organic tomato production. Progress on specific goals:

1. Monitor and analyze soil health indicators (including biological, chemical, and physical soil measurements) and nutrient release changes.
 - 1.1. Nutrient levels were similar among all plots during plot setup and throughout the first year. Nitrogen dynamics are different between rotations, but other nutrients and SOM have not shown significant differences.
 - 1.2. Physical soil health parameters have been estimated for baseline status. Infiltration tests revealed alfalfa plots have a much higher saturated hydraulic conductivity than the annual rotation, although repetition with a larger sample size is required for robust conclusions to be made. Aggregate stability of near-surface soil has so far been similar; another test is planned using second-year soil.
 - 1.3. Soil incubation experiments revealed a difference in potentially mineralizable carbon (PMC-24h) between rotations, with Alfalfa soil mineralizing 25% less carbon. However, nitrogen mineralization was not different between any treatments. Additionally, nitrogen mineralization was not correlated to carbon mineralization in the alfalfa soil, but significantly so in the cover crop soil. Potential substrate limitations to mineralization rates was explored by measuring soil and root total carbon and nitrogen (TC and TN), revealing 10% lower TC and TN in alfalfa, likely due to lower compost inputs. Soils from plots that had been grazed resulted in slightly lower rates of carbon mineralization in both rotation treatments, though differences were smaller than the observed rotation effect. Another round of incubation experiments is planned to further explore these results and increase our confidence in the emerging patterns.
 - 1.4. Biological parameter tests (Microbial Carbon, PLFA, Enzyme activity) are in preparation for soils from the first and second years. Equipment failures are delaying some analyses.
2. Evaluate the effect of winter livestock grazing during the transition period on root growth, soil health indicators, and nutrient release.
 - 2.1. Throughout the first winter and summer, we found no significant effects of crop or grazing treatments on soil nutrient availability and organic matter content. Second year grazing included the alfalfa plots. Analysis of soil fertility is ongoing, and crop yields are currently being calculated.
 - 2.2. Grazing caused a slight reduction (~10%) in certain soil health parameters, including total nitrogen, total carbon, and potentially mineralizable carbon.
 - 2.3. Root biomass was slightly higher during spring in the annual rotation compared to Alfalfa. As Alfalfa has a slower growth rate in winter, that outcome was expected. Second year alfalfa root biomass was up to 10-fold higher than first-year alfalfa. Root biomass of second year alfalfa and summer cover crops is currently being analyzed for biomass and carbon and nitrogen content.
3. Compare weed biomass, weed community composition, and the weed seedbank during the transition period.
 - 3.1. Data on weed seedling densities and community composition has been analyzed. Data on weed cover and biomass has been recorded and analyzed for the spring and most of the summer season. Whole community and species-specific seedling densities were unrelated to late-season weed biomass, with most biomass being produced by two species with sparse seedling densities (Mustard and Lambsquarters). Weed cover and abundance, measured in quadrates in summer 2021, was unaffected by grazing. One exception seems to be a reduction in abundance of the weed Tumble pigweed in grazed plots. This result stands contrary to data collected in another grazing study, showing increased broadleaf weed biomass, mostly pigweeds, in grazed plots.
 - 3.2. The weed seedbank, as tested by a field survey of sprouting weeds and germination in controlled greenhouse assays, showed no difference among any treatment combinations.
 - 3.3. The impact of sheep grazing on winter and spring weed cover could not be isolated or assessed thoroughly, as the WCC and the second-year alfalfa effectively controlled weeds. Summer weed cover, although significantly limited by forage crops, did not differ among treatments. Summer weed community composition is currently under analysis. Preliminary observations from a parallel experiment (run by Gaudin's lab) shows potential for spring grazing to reduce weed pressure during organic tomato cultivation. A survey in that field revealed lower abundance but similar biomass of Barnyardgrass, a summer annual weed common in organic fields. We are running a germination assay, using an improved protocol, to assess the effect of grazing on germination and early growth of Barnyardgrass.
 - 3.4. Weed cover and biomass significantly reduced organic alfalfa yield in the first year (up to 40% below conventional yield), with some slight residual effect showing in second-year harvests as well. This highlights one potential drawback of using alfalfa for the transition period. However, weeds could be better managed by introducing an earlier first cut of the alfalfa or by including grazing during the establishment year.
4. The land use systems (LUS) model is being constructed by collecting information on expected costs and revenue associated with application of different management scenarios. As is the case for many researchers in the past 2 years, the COVID-19

pandemic impacted our research in several ways, including the inability to hold planned outreach activities due to the cancellation of in-person programming. We do have plans to present organic alfalfa system results at an outreach event in November 2021, in collaboration with UC Davis Extension forage researchers. Additionally, we held a webinar last winter for California organic tomato growers and other practitioners ("Alternative management in Organic Tomato Production", January 5, 2021), to disseminate project results. We have so far been communicating with other researchers conducting studies on organic agriculture and alfalfa management, working to make our data useful for a wide audience. These include members of Prof. Amelie Gaudin's lab in UC Davis, who are conducting a parallel experiment with sheep grazing in established organic tomato fields. Their results will shed light on our expected outcomes with regard to the future tomato production in our plots, while our results combined with theirs will provide a larger data base and more case studies for those interested in the transition to organic production **PUBLICATIONS (not previously reported):** 2020/09 TO 2021/08 No publications reported this period.

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Evaluation of Integrated Bacterial Disease Management Options for Organic Onion Production in Southeastern and Northcentral United States

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NON-TECHNICAL SUMMARY

Center rot has emerged as a chronic problem in number of onion growing regions (conventional and organic) in the United States including Georgia and Michigan, and has been responsible for significant pre- and post-harvest losses in yield and quality. The primary emphasis of the proposed project is to advance technologies and outreach to promote the transition to organic onion production in two productive regions, southern and northcentral U.S. Our integrated approach focuses on minimizing inoculum sources (weeds, thrips) through microbial control, weed management, and biological control provisioning to form an environmentally sound package for organic onion growers. We also intend to compare the relative impact of organic treatments and management tactics on the biodiversity of parasitoids and predators. Additionally, we will conduct a cost-benefit analysis to assess how the new management strategy will result in increased profits. In addition, we will involve a "Stakeholder-Advisory Panel" who will independently evaluate the performance of the project during the project duration. We envision that the outcomes of this project will improve center rot management strategies with an "integrated" approach and will ensure the sustainability and profitability of organic onion in the U.S. The current project is in line with the stakeholder's need/input and the ORG RFA priorities: understanding of weeds, pests and disease dynamics in organic system (priority 1); and overcome barriers to organic transition by developing practical information and tools for producer use (priority 2).

OBJECTIVES

Objective 1: Evaluate seed, microbial and chemical management options for *Pantoea* sp. and onion thrips in an organic production system. Sub-objective 1a: Screen for physical and biological seed treatments for the control of seedborne infection by *Pantoea* sp. in onion. Sub-objective 1b: Evaluate options to increase efficacy of the OMRI certified microbial- compounds for the management of *Pantoea* sp. under field conditions. We will also evaluate a thrips management program with OMRI-labeled insecticides in combination with the microbial treatments for *Pantoea* sp. Objective 2: Determine the impact of weed control on *Pantoea* sp. incidence/severity, insect vectors

and natural enemies under field conditions. Objective 3: Integrate management tactics, conduct economic analysis and implement outreach program. Objective 4: Project evaluation by the stakeholder advisory panel.

APPROACH

In objective 1a we will evaluate physical and biological seed treatments using *P. ananatis* as a model system. Since, *P. ananatis* is closely related to *P. agglomerans* and also a part of the center rot complex, information generated for one species can possibly be used for other *Pantoea* sp. Seed treatments: Artificially infested onion seeds will be used for the evaluation of physical, OMRI labeled-chemical and biological seed treatments against *P. ananatis*. In order to generate infested seeds, onion seeds will be vacuum infiltrated with a suspension containing *P. ananatis* (strain: PNA 97-1) (108 colony forming units/ml) and air-dried as described earlier (29). A total of three independent trials will be conducted. Each independent trial will use a lot of 10,000 onion seeds artificially infested with the pathogen. Four replicated samples of 250 seeds each per seed treatment-type will be used. Infested onion seeds will be treated with physical, chemical and biological seed treatments as listed in Table 4. For chemical seed treatments, the OMRI labeled copper bactericides as suggested by the 2018 ORG review panel (Nordox 75WG, Plant Food Company Inc., NJ and Kalmor, OHP Inc., SC) will be utilized. Infested seeds not treated physically or chemically or biologically will serve as negative controls. The seed treatments will be evaluated based on their ability to eradicate *P. ananatis* inoculum from onion seeds without affecting seed germination. To achieve this, percent seed germination, percent reduction in *P. ananatis*-infested seeds and percent reduction in seed-to-seedling transmission of center rot will be determined for each seed sample after treatment (physical and biological). In objective 1b, We will evaluate *P. ananatis* as a model system for center rot in both Georgia and Michigan. Our plan is to utilize OxiDate 2.0 (Biosafe Systems; East Hartford, CT) as a niche-clearing treatment for the onion foliage and determine the effect of this sterilization step on subsequent colonization by foliar microbial-control agents. This mode of application will promote efficient colonization in absence of any competitor on phyllosphere. Onion foliage well colonized by microbial-control can competitively exclude any incoming foliar pathogen including *P. ananatis*. We will use OxiDate 2.0 (at labeled rate; one application) as a niche-clearing treatment for the onion foliage 21 days after transplanting. Two OMRI-certified bacterial microbial-control agents, Serenade ASO (3g/liter) and BlightBan (1g /liter) will be assessed for their center rot control abilities. After two days of microbial-control application, onion plots except untreated check plots, will be spray inoculated with 1×10^4 CFU of *P. ananatis* (PNA 97-1). In Georgia and Michigan, in addition to the treatments listed above, we will test an onion thrips management program comprising of a rotation of OMRI approved spinosad and neem oil extract applied when thrips populations in the field exceed 0.5 thrips per leaf threshold. We will test the insecticide program in combination (tank-mixed) with the microbial treatments listed above and alone. All products will be applied using a CO2 backpack sprayer and a broadcast boom equipped with three XR8003 flat-fan nozzles (TeeJet® Technologies, Wheaton, IL) with the outer nozzles angled towards the center of the plot, calibrated at 50 psi and delivering 467.5 L/ha. In objective 2, we will evaluate if mechanical and hand-weeding can reduce *Pantoea* infection. We added a hand-weeded treatment to this proposal to ensure that we provide information for our smaller organic onion growers who do not use Einböck tine weeder (Aerostar Tined Weeder; Einböck GmbH & CoKG, 4751 Dorf an der Pram, Austria). We also aim to document natural enemy associations with thrips in relation to different proposed weed treatments (mechanical and hand-weeding). Three weed control treatments will be evaluated to determine the effects of weed presence and method of weed control on onion yield and *P. ananatis* incidence. We will rely on natural infection as our previous observations indicate that most of the weeds in onion pathosystem harbor *Pantoea* sp. as an epiphyte. In objective 3, the one or two best performing treatments from each objective above will be utilized where we will integrate approaches to form an organic management package for onions. Two strategies will be optimized and tested as IPM strategy combination #1 (Best performing treatments selected from above objectives) and IPM strategy combination #2 (2nd best treatments selected from above objectives) (Fig. 9). These treatments will be compared to an organic onion grower's standard treatment program for disease control and an untreated control. This objective will be conducted at two certified organic fields each in Georgia and Michigan. We will extend our information at local and regional meetings focusing on onion production, and disease management after the first year in GA and MI. We will also conduct economic analysis of the improved management package compared to the existing organic transitioning or organic growers' standard. In objective 4 project evaluation will be conducted by the stakeholder advisory panel. **Progress** 09/01/19 to 08/20/22 **Outputs** Target Audience: Primary audience of the project is the organic onion producers in Georgia and Michigan. This includes organic onion producers, packers, shippers, agronomists, crop consultants, farm managers, field workers, seed companies, and dealers; and onion storage and shipping/transport personnel and companies. Targets also include postdocs, graduate students, and undergraduate students who receive training in microbiology, molecular genetics, genomics, and scientific communication. Changes/Problems: For Co-PIs Hausbeck and Szendrei, there appears to have been a recent shift in foliar disease pressure from bacterial disease caused by *Pantoea* spp. to fungal diseases with *Stemphylium* leaf blight and anthracnose being the most prevalent fungal pathogens. In addition to not detecting

symptomatic foliar caused by *Pantoea* spp. or other bacteria in our 2020 and 2021 field plots we also noted no bacterial rot in the harvested onion. This is in contrast to the disease pressure when this grant was created and that time bacterial diseases were very prevalent. Over the years we have observed that some diseases can be cyclical. Co-PIs Hausbeck and Szendrei switched focus to the two primary fungal diseases, *Stemphylium* leaf blight and anthracnose, for the field experiment. Co-PI Szendrei switched to working with Anthracnose in laboratory bioassays. What opportunities for training and professional development has the project provided? Five graduate students (3 at UGA and 2 at MSU) and one post-doctoral research associate have been hired to work on various aspects of the project. Extension agents and consultants were also trained on the symptoms and management of bacterial diseases on onion. PI Dutta and Co-PIs Grey, Schmidt, Szendrei and Hausbeck provided training for graduate students in both field- and laboratory-based agricultural scientific techniques. Field-based techniques include establishing and evaluating field biopesticide efficacy trials, field plot design, field plot maintenance, fungicide application, disease diagnostics, disease evaluation, and yield assessments. Laboratory-based techniques included the isolation and morphological identification of disease-causing organisms, data analysis, statistics, and the preparation of presentations, technical reports, and manuscripts. Co-PI Hausbeck provided training for graduate students in both field- and laboratory-based agricultural scientific techniques. Field-based techniques include establishing and evaluating field biopesticide efficacy trials, field plot design, field plot maintenance, fungicide application, disease diagnostics, disease evaluation, and yield assessments. Laboratory-based techniques included the isolation and morphological identification of disease-causing organisms, data analysis, statistics, and the preparation of presentations, technical reports, and manuscripts. Co-PI Szendrei provided training and education for a graduate and two undergraduate students. The graduate student learnt how to set up and assess onion thrips trials in the field, and how to analyze and present data in several different formats. The graduate student also learnt to maintain disease and thrips colonies in the lab and use these in bioassays. The undergraduate students learnt how to identify insects and plant diseases, assess experiments, and collect data. How have the results been disseminated to communities of interest? PD Dutta, Co-PI Grey, Schmidt extended these information via several county extension meetings, regional (Southeast Fruit and Vegetable Conference) and national meetings (American Phytopathological Society, Entomological Society of America). All together 15 county extension meetings were conducted in Georgia in 2021-2022 season along with Vidalia Onion field days (n=2) and two production meetings. We also published several popular press articles, peer-reviewed articles and abstracts for disseminating the information to the stakeholders. Co-PIs Hausbeck and Szendrei implemented extension programming to educate growers, industry stakeholders, extension educators (MSUE), and representatives from the Michigan Department of Agriculture and Rural Development (MDRAD) on managing thrips and foliar onion diseases in Michigan. Eight extension presentations were given at the Onion Committee Meeting (12 attendees: growers, MDARD, MSUE), Great Lakes Exposition (103 attendees (2020) 46 attendees (2021): growers, industry stakeholders), and Vegetable and Root Crop Virtual Field Day (39 attendees: growers, industry stakeholders), Michigan Onion Committee Twilight Meeting (45 attendees: growers, industry stakeholders), and Michigan Onion Committee Research Update (20 attendees). A virtual walk-through of research plots was integrated with research results and posted to YouTube (35 views). Contributions to a draft of the Pest Management Strategic Plan for onions in Michigan were made. Four extension publications were published in Vegetable Grower News and Onion World and two conference proceedings were written for a grower audience at the Great Lakes Exposition. Two informational posters were presented at the American Phytopathological Society Annual Meeting and the Great Lakes Exposition. Two peer-reviewed journal articles were submitted, one was published, and the other is under review. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported **Impacts** What was accomplished under these goals? Objective 1: In Georgia, trials were conducted in 2021 during onion growing seasons in a certified organic field to evaluate if pre-application of a micro-biocide (OxiDate 2.0) on foliar surfaces of onion could enhance the colonizing ability of subsequently applied BCAs (BlightBan (*Pseudomonas fluorescens*) and Serenade (*Bacillus subtilis*)) and improve their effectiveness in managing center rot. We assessed the foliar disease severity on onion foliage and center rot incidence in harvested bulbs after storing the bulbs in cold storage for a month. We also conducted microbiome composition of onion foliage was evaluated over time for BlightBan treated plots. The BCAs and OxiDate 2.0 significantly interacted and affected the area under disease progress curve (AUDPC). OxiDate 2.0 pre-treated BlightBan plots did not differ significantly from BlightBan treated plots in reducing AUDPC. Similarly, although OxiDate 2.0 pre-treatment prior-to Serenade application seemed to significantly reduce the foliar AUDPC in 2020, the results was inconsistent in 2021 trial. The incidence of center rot in onion bulbs followed the similar trend as OxiDate 2.0 pre-application before BCAs did not seem to significantly reduce the incidence of bulb rot as compared to the stand-alone treatment with BCAs. Moreover, our microbiome study showed that the pre-application of OxiDate 2.0 potentially favored better colonization by *P. fluorescens* in BlightBan; however, this colonizing ability is transient as the populations of *P. fluorescens* were drastically reduced at the end of the growing season. Interestingly, *Pantoea* species were widely prevalent in onion foliage throughout the season. Although, we did not evaluate the sole effect of OxiDate 2.0 in influencing the microbial community structure, the fact that stand-alone OxiDate 2.0 treatment had similar efficacy as BCAs in managing

center rot demands further investigation into its potential activity in foliar surface of onion. During the 2021 growing season in Michigan, Co-PI Szendrei conducted a small plot randomized trials at a collaborating grower's farm in mid-Michigan to determine the efficacy of using different thrips thresholds to start Neemix applications for onion thrips control. Neemix is an organic insecticide registered for use in onions. Onion thrips pressure was similar across treatments with none of the tested thresholds providing suppression beyond economic injury level. We also tested the effect of plot weediness on thrips pressure and determined that unweeded plots had significantly fewer thrips than weeded plots. Unweeded plots also had significantly more natural enemies than weeded plots. In bioassays we examined the effects of varying onion plant health status (control; mock inoculated with *Colletotrichum coccodes*), artificially injured, artificially injured + inoculated-symptomatic, and inoculated-symptomatic) on onion thrips preference in a laboratory setting using a four-choice test. After 13 days, the number of adult and larval onion thrips were recorded on each plant. Co-PIs Szendrei and Hausbeck conducted field trials with a grower cooperater in Hamilton, MI in 2020 and 2021 to evaluate biorational compounds for the management of *Pantoea* sp. and thrips under field conditions. The efficacy of Neem and Entrust - OMRI approved thrips products - were compared in combination with Kocide which is a general fungicide/bactericide. In 2021, conventional insecticide and fungicide treatments were added as control. *Stemphylium* leaf blight was the primary foliar pathogen observed during the trials; disease from *Pantoea* spp. were not observed. Premature leaf death resulting in a mid-season loss of green leaf tissue is typical of *Stemphylium* leaf blight and the percentage of green tissue was assessed weekly Jul - Aug. In 2020, some OMRI approved thrips products (Entrust, Entrust + Kocide 3000-O, and Neem Oil + Kocide 3000-O) had less disease than the untreated control and the fungicide/bactericide (Kocide 3000-O). Thrips numbers were statistically similar across all treatments with pesticides. However, thrips assessment did not overlap the period where disease pressure was the greatest. In 2021, none of the OMRI approved thrips products reduced disease compared to the untreated control. Control treatments with conventional insecticide had significantly less disease than the untreated and when combined with conventional fungicide was the most effective strategy. Overall, managing thrips appears to help reduce *Stemphylium* leaf blight, but the OMRI approved products tested were not effective enough to realize the benefit.

Objective 3; integrated management tactics: Based on year 1 and 2 trials, we evaluated an integrated trial where onion plots were either hand-weeded throughout the season or mechanically weeded using tune weeder for first 8-weeks of transplanting followed by hand-weeding rest of the season in a certified organic field. An optimized bacterial management program from objective 1 (BlightBan and Nordox) was overlaid on top of these treatments. Data indicate that weed control and disease severity in onion bulbs due to center rot was not significantly different for the two integrated treatments. Also, we did not observe any qualitative and quantitative changes in the diversity of insect predators and parasitoids. These results indicate that tine-weeding and hand-weeding is as effective as a labor-intensive hand-weeding only treatment.

Economics of integrated management: Co-PI Colson conducted an economic analysis comparing hand weeding vs. hand weeding and tine weeding was conducted employing market price data and results from the experimental field trials including yields and labor productivity rates. Based upon the experimental results, the more labor-intensive field management strategy (hand weeding) is more profitable for growers to adopt if per-acre weeding productivity is sufficiently high. At lower productivity rates, the less labor-intensive strategy employing a tine weeder is more profitable for growers. Results indicate given the experimental plot yields, at a baseline onion price of \$17.00 per 40lb. box and an hourly labor rate of \$14, hand weeding is a more profitable strategy for a producer to adopt if the season-long per-acre increase in labor required is less than 175 hours more compared to combined hand weeding and tine weeding. For acreage requiring greater hours of labor hand weeding, there is an economic return to adopting tine weeding. As there is heterogeneity in weed intensity and labor productivity rates, sensitivity analysis was conducted to estimate the break-even productivity rates over a range of onion prices and hourly labor rates. Results indicate during years of low onion prices or high wage rates, adoption of tine weeding is more likely to be profitable for growers while years with high onion prices or low wage rates, adoption of hand weeding only is more likely to be profitable.

Objective 4: Stakeholder advisory panel met in January 2022 (virtually) where PD and Co-PIs shared the results of year 2 trial and received inputs for year 3. Based on the inputs received from the advisory panels, slight modification in the treatments were made in year 3 trials both in Georgia and Michigan.

****Publications**** - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Szendrei, Z. Venturing into Organic Thrips Control in Onions, Great Lakes Expo Fruit, Vegetable, and Farm Market, Grand Rapids, MI, 8 Dec. - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: De Jesus, H., K. Cassity-Duffey, M. Cabrera, A. da Silva, B. Dutta, and T. Coolong. 2022. Influence of soil temperature on plant available nitrogen from organic fertilizers. SRASHS. Feb. 12, 2022, New Orleans, LA - Type: Other Status: Published Year Published: 2022 Citation: Dutta, B., Grey, T., and Schmidt, J. 2022. Neglecting weeds can lead to late-season disease in organic onions. Specialty Crop News. March 2022. - Type: Other Status: Published Year Published: 2021 Citation: DevKumar, G., Kvitko, B.H., and Naikare, H. 2021. Studying Salmonella contamination in onion. Vegetable and Specialty Crop News, August 2021. - Type: Journal Articles Status: Under Review Year Published: 2022 Citation: Constancio, N., Higgins, D., Hausbeck, M., and Szendrei, Z. 2022. Onion thrips (Thysanoptera: Thripidae) host

plant preference and performance are mediated by a plant disease. Environmental Entomology - Type: Journal Articles Status: Published Year Published: 2021 Citation: Agarwal, G., Gitaitis, R.D., and Dutta, B. 2021. Pan-genome of novel *Pantoea stewartii* subsp. *indologenes* reveals genes involved in onion pathogenicity and evidence of lateral gene transfer. *Microorganisms* 9: 1761. <https://doi.org/10.3390/microorganisms9081761>. - Type: Journal Articles Status: Published Year Published: 2021 Citation: Agarwal, G., Choudhary, D., Stice, S.P., Myers, B.K., Gitaitis, R.D., Venter, S.N., Kvitko, B.H., and Dutta, B. 2021. Pan-genome-wide analysis of *Pantoea ananatis* identified genes linked to pathogenicity in onion. *Frontiers in Microbiology* <https://doi.org/10.3389/fmicb.2021.684756>. - Type: Other Status: Published Year Published: 2022 Citation: Constancio, N., Higgins, D., Hausbeck, M., and Szendrei, Z. 2022. Two challenges, one strategy: onion thrips and *Stemphylium* leaf blight management. *Onion World* **Progress** 09/01/20 to 08/31/21 **Outputs** Target Audience: Primary audience of the project is the organic onion producers in Georgia and Michigan. This includes organic onion producers, packers, shippers, agronomists, crop consultants, farm managers, field workers, seed companies, and dealers; and onion storage and shipping/transport personnel and companies. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? Five graduate students (3 at UGA and 2 at MSU) and one post-doctoral research associate have been hired to work on various aspects of the project. Extension agents and consultants were also trained on the symptoms and management of bacterial diseases on onion. PI Dutta and Co-PIs Grey, Schmidt, Szendrei and Hausbeck provided training for graduate students in both field- and laboratory-based agricultural scientific techniques. Field-based techniques include establishing and evaluating field biopesticide efficacy trials, field plot design, field plot maintenance, fungicide application, disease diagnostics, disease evaluation, and yield assessments. Laboratory-based techniques included the isolation and morphological identification of disease-causing organisms, data analysis, statistics, and the preparation of presentations, technical reports, and manuscripts. How have the results been disseminated to communities of interest? The results were disseminated through various means like journal articles, popular press articles, extension bulletins, conferences (state, regional and national) and county extension meetings in Georgia and Michigan. What do you plan to do during the next reporting period to accomplish the goals? In year 3 we will repeat the trial where we will integrate management tactics, conduct economic analysis and implement outreach program. We will also conduct project evaluation by the stakeholder advisory panel. **Impacts** What was accomplished under these goals? Sub-objective 1a: PI Dutta and collaborator Petkar evaluated the efficacy of biological and physical seed treatments for onion against seedborne *Pantoea* spp. infection. Based on replicated trials in 2021, it was observed that onion seeds treated with Serenade (3g/L; exposure time: 30 or 60 minutes) or hot water (at 55°C for 15 minutes) or Nordox 75 WG (5.5g/L; exposure time: 15 minutes) was able to significantly reduce seed-to-seedling transmission of *Pantoea* spp. without impacting seed germination compared with non-treated check and other physical, chemical and biological seed treatments under various concentration, exposure temperature and duration. Sub-objective 1b: A field trial was conducted in an OMRI-certified field at UGA, Tifton in Spring 2021. This field trial evaluated options to increase efficacy of the OMRI-certified microbial-compounds for the management of *Pantoea* sp. in onion. The results indicate that the area under disease progress curve (AUDPC) values were significantly higher for plots that were not treated with any bio-control agents (BlightBan or Serenade ASO) or OMRI-certified insecticide program (Neem oil and Spinosad rotation) or Oxidate 5.0 alone treatment compared with other treatments. The AUDPC values for the treatments with biocontrol agents alone or along with Oxidate 5.0 or with an insecticide program were not significantly different from each other. Application of OMRI-certified insecticide program significantly reduced total thrips count compared with no- OMRI-certified insecticide treatment. Foliar microbial application significantly did not significantly reduce center rot incidence in bulb ($P=0.233$) when assessed after 30 day of harvest. Although some trends were observed where plots with biocontrol agents had considerably lower center rot bulb incidence compared with no bio-control treatments. Foliar application of Oxidate 5.0 did not seem to improve the efficacy of biocontrol agents (Serenade and BlightBan) when bulb rot incidence was assessed. Based on the two-years of field trials in Georgia, we conclude that application of Oxidate 5.0 prior to bio-control applications did not improve their efficacy in terms of reducing foliar and bulb center rot. The OMRI-certified insecticide program used in this project for thrips control alone is not effective for two consecutive years for reducing foliar as well as bulb center rot. Co-PIs Szendrei and Hausbeck conducted a field trial with a grower cooperator in Hamilton, MI in 2020 to evaluate biorational compounds for the management of *Pantoea* sp. and thrips under field conditions. The efficacy of Neem and Entrust - OMRI approved thrips products - were compared in combination with Kocide-3000-O, which is a general fungicide/bactericide. Pesticide applications were made weekly Jun-Aug and thrips numbers were assessed visually weekly. *Stemphylium* leaf blight was the primary foliar pathogen observed during the trial; disease from *Pantoea* spp. were not observed. Premature leaf death resulting in a mid-season loss of green leaf tissue is typical of *Stemphylium* leaf blight and the percentage of green tissue was assessed weekly July-August. Some OMRI approved thrips products (Entrust, Entrust + Kocide 3000-O, and Neem Oil + Kocide 3000-O) had less disease than the untreated control and the fungicide/bactericide (Kocide 3000-O). Objective 2: Co-PI Grey conducted a field experiment that was planted at the UGA, Tifton certified organic production area that was tilled

by moldboard plowing then smoothed with a rotary tiller to form six-foot wide beds. Organic onion bulb onions sets were hand transplanted in Dec 2020. Treatments application and data collection began after planting and continued till May 2020. Treatments included a nontreated control (weed infested), plots that were maintained clean of weeds by hand for eight weeks, and mechanical tine cultivation occurring two, four, six, and eight times with additional hand weeding twice, and four times. The intent was to evaluate mechanical and hand weeding at distinct timings: 2, 4, 6, 8, 10, 12, 14, and 16 weeks after planting. Wild radish was the predominant weed species, but cutleaf evening primrose and swinecress were also present. Over time, wild radish became the dominant species and suppressed organic onion growth. Hand weeding did not provide adequate season long weed control. Though the addition of cultivation to hand weeding did slightly increase weed control, it was still not adequate for organic control. Cultivation alone provided the greatest season long weed control with cultivation occurring 4 or 8 times. Overall the objective of this research was achieved as tine weeding did reduce the number of weeds and when cultivated up to eight times. However, further research is required to establish how further tine weeding could be used to mitigate other weed species. Monitoring thrips and natural enemies, and predators: During this project period, Co-PI Schmidt conducted an experiment to assess different levels of mechanical weeding on weed control, insect pressure and natural enemy populations in an organically certified experimental field. We deployed and collected sticky cards and pitfall traps on three dates prior to harvesting in each year. Sampling began on the week following the final cultivation treatment for each plot. A total of 1 pitfall and one sticky card were deployed and collected for a total of 72 for each year. Currently, we have processed all the sticky cards (year 2020 and 2021 of the project), and have pitfall traps transferred to ethanol for further processing. Overall, much higher thrips pressure was observed in 2020 as compared to 2021. While there were treatment differences in 2020, these were not present in 2021, likely a result of not having many thrips in 2021. Another interesting pattern observed in 2020, is that fewer thrips were observed in treatments that received no hand weeding, and a correspondingly higher abundance of parasitoid wasps were observed. Objective 3: Integration of management tactics was conducted in year 2 where three different management options into one integrated treatment were evaluated; weeding (mechanical and hand), foliar bio-control agent (Serenade ASO) and OMRI-certified thrips management program (spinosad and neem oil). This integrated treatment was compared with the grower's standard practice (hand weeding six times, foliar application of Nordox at a 14 day interval and application of neem oil for thrips control). Although, our data did not indicate any significant differences between integrated treatment vs. grower's standard, we observed considerable decrease in final foliar disease severity and AUDPC value with the integrated treatment than with the grower's standard treatment. PI Dutta gave updates on onion bacterial disease management options in 5 different county meetings (virtually) in Georgia, January-March 2021 (n=230 attendees in total). Co-PI Hausbeck implemented extension programming to educate growers, industry stakeholders, extension educators (MSUE), and representatives from the Michigan Department of Agriculture and Rural Development (MDRAD) on foliar onion diseases in Michigan. Three extension presentations were given at the Onion Committee Meeting (12 attendees: growers, MDARD, MSUE), Great Lakes Exposition (103 attendees: growers, industry stakeholders), and Vegetable and Root Crop Virtual Field Day (39 attendees: growers, industry stakeholders). A virtual walk-through of research plots was integrated with research results and posted to YouTube (20 views). Objective 4: Stakeholder advisory panel met in January 2021 (virtually) where P1 and Co-PIs shared the results of year 1 trial and received inputs. Based on the inputs received from the advisory panels, slight modification in the treatments were made in year 2 trials both in Georgia and Michigan. **Publications** - Type: Journal Articles Status: Accepted Year Published: 2021 Citation: Koirala, S., Zhao, M., Agarwal, G., Stice, S., Gitaitis, R., Kvitko, B., and Dutta, B. 2021. Identification of two novel pathovars of *Pantoea stewartii* subsp. *indologenes* affecting *Allium* sp. and millets. *Phytopathology* (in press) - Type: Journal Articles Status: Published Year Published: 2021 Citation: Stumpf, S., Leach, L., Srinivasan, R., Coolong, T., Gitaitis, R., and Dutta, B. 2021. Foliar chemical protection against *Pantoea ananatis* in onion is negated by thrips feeding. *Phytopathology* 111: 258-267. - Type: Journal Articles Status: Published Year Published: 2021 Citation: Higgins, D.S., Szendrei, Z., and Hausbeck, M.K. 2021. Evaluation of biorationals for control of *Stemphylium* leaf blight on onion, 2020. *Plant Disease Management Reports*, 15:V049. - Type: Journal Articles Status: Other Year Published: 2021 Citation: Lennon KM, Dutta B, Johnson C, Grey T, Thompson M, Arabuli T, Szendrei Z, Schmidt JM. 2021. Weed cultivation and bactericides influence thrips abundance in an organic onion system. *Crop Protection* (in-prep). - Type: Other Status: Published Year Published: 2021 Citation: Dutta, B. 2021. Onion disease management in Georgia. *Vegetable and Specialty Crop News*, February 2021. - Type: Other Status: Published Year Published: 2021 Citation: Dutta, B., and Gitaitis, R.D. 2020. Disease quiz II. *Onion World Magazine*. January 2021. - Type: Other Status: Published Year Published: 2020 Citation: Hausbeck, M. 2020. The Next Chapter in Onion Foliar Disease. *Onion World*: September/October 2020: 16-17. - Type: Conference Papers and Presentations Status: Published Year Published: 2020 Citation: Lennon KM, Dutta B, Johnson C, Thompson M, Arabuli T, Szendrei Z, Schmidt JM. 2020. Interactions between weed cultivation and bactericides on thrips abundance, natural enemies, and disease severity in an organic onion system. *Entomological Society of America* (virtual meeting Nov. 11-25; oral on-demand) - Type: Conference Papers and Presentations Status: Other Year Published: 2021 Citation: Management of bacterial bulb rot with

growth-stage targeted copper management in onion. Cornell University Cooperative Extension (Orange County, NY)

PROGRESS

2019/09 TO 2020/08 Target Audience: Primary audience of the project is the organic onion producers in Georgia and Michigan. This includes organic onion producers, packers, shippers, agronomists, crop consultants, farm managers, field workers, seed companies, and dealers; and onion storage and shipping/transport personnel and companies. Changes/Problems: Due to COVID-19 related restrictions, we were not able to conduct objective 1 a (seed treatment). We will start objective 1a in October, 2020. Also, onion plantings were delayed in MSU due to COVID-19 related issues, hence we were not able to share results from year 1 of the project (MSU portion). However, co-PIs at MSU are currently conducting field trials as per the project and will share results in our next reporting period. What opportunities for training and professional development has the project provided? Five graduate students (3 at UGA and 2 at MSU) have been hired to work on various aspects of the project. Extension agents and consultants were also trained on the symptoms and management of bacterial diseases on onion. How have the results been disseminated to communities of interest? Results were disseminated through county extension meetings, conference presentations (National Onion Association and Southeast Fruit and Vegetable Grower Conference), publishing extension bulletins on bacterial diseases of onion and their potential management options. What do you plan to do during the next reporting period to accomplish the goals? We will repeat objective 1-4 and will also find opportunities to extend our findings through various platforms to the stakeholders. We will also prepare journal articles, extension bulletins and newsletters for our stakeholders.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? A field trial was conducted in an OMRI-certified field at UGA, Tifton in Spring 2021. This field trial evaluated options to increase efficacy of the OMRI-certified microbial-compounds for the management of *Pantoea* sp. in onion. A randomized complete block design with four replications of each treatment were utilized. Plots consisted of 5-foot-wide beds containing four rows of onion plants with 4-inch within row spacing. Plots were 20-feet in length with 10 foot borders within adjacent plots within a bed. A late-maturing, highly susceptible variety (Sweet Jasper) to center rot was used. We used OxiDate 5.0 (at labeled rate; one application) as a niche-clearing treatment for the onion foliage 21 days after transplanting. Two OMRI-certified bacterial microbial-control agents, Serenade ASO (3g/liter) and BlightBan (1g /liter) were assessed for their center rot control abilities. After two days of microbial-control application, onion plots were spray inoculated with 1×10^4 CFU of *P. ananatis* (PNA 97-1). Plots neither sprayed with microbial-compounds or *P. ananatis* served as non-treated non-inoculated treatment. In addition to the microbial compounds, we also tested an OMRI-labeled insecticide program (Neem oil and Spinosad rotation; used when thrips populations in the field exceeded 0.5 thrips per leaf threshold) in combination with the microbial treatments listed above and alone. Disease severity data for each plot was collected at two-week intervals starting from 14-days after pathogen inoculation. A total of four disease assessments were made and at the end of the season an area under disease progress curve (AUDPC) was calculated for each treatment. Final foliar disease severity and AUDPC were significantly higher for plots that were not treated with any microbial compounds or OMRI-labeled insecticide program but inoculated with *P. ananatis*, and insecticide program only treatment compared with other treatments. Among the treatments, AUDPC for the treatment that had Serenade along with an OMRI-labeled insecticide program or Oxidate 5.0 along with an OMRI-labeled insecticide program was significantly higher than the Oxidate 5.0+Serenade treatment. Significant differences among treatments with respect to AUDPC values were not observed for the Serenade only or the BlightBan+OMRI-labeled insecticide program or Oxidate 5.0+BlightBan+OMRI-labeled insecticide program; Oxidate 5.0+Serenade+OMRI-labeled insecticide program or Oxidate 5.0 only or Oxidate 5.0+BlightBan or BlightBan only. Application of OMRI-labeled insecticide program significantly reduced total thrips count compared with no-OMRI-labeled insecticide treatment. Foliar microbial application significantly reduced center rot incidence in bulb ($P=0.003$) when assessed after 30 day of harvest. The interactive effect of niche clearing treatment (Oxidate 5.0) prior to foliar microbial application (Serenade or BlightBan) on center rot bulb incidence was significant ($P=0.006$). The interactive effect of foliar microbial treatment*OMRI-labeled insecticide program or Oxidate 5.0* OMRI-labeled insecticide program or Oxidate 5.0*foliar microbial treatment*OMRI-labeled insecticide program was not significant ($P<0.05$). Insecticide program or Oxidate 5.0 only treatment did not significantly reduce center rot bulb incidence. Bulb incidence of center rot was significantly reduced for Serenade only or BlightBan only treatments compared with no-foliar microbial treatments. Foliar niche clearing treatment (Oxidate 5.0) along with Serenade or BlightBan application significantly reduced center rot bulb incidence compared with only Oxidate 5.0 treatment. For objective 2, field experiments were planted at the Tifton GA certified organic field that was tilled by moldboard plowing equipment

then smoothed with a rotary tiller to form 6-foot wide beds. Organic onion bulb onions sets were hand transplanted in Dec 2019. Treatments application and data collection began after planting and continued till May 2020. Treatments included a non-treated control (weed infested), plots that were maintained clean of weeds by hand for eight weeks, and mechanical tine cultivation twice, four times, and mechanical tine cultivation with additional hand weeding twice, and four times. The intent was to evaluate mechanical and hand weeding at distinct timings: 2, 4, 6, and 8 weeks after planting. The experimental design was a randomized complete block with four replications and plots had four onion rows per bed 20 feet long. Injury ratings for physical injury were visually estimated during the course of the study and no negative aspects were recorded as stand counts remained consistent up to late Jan. Wild radish (*Raphanus raphanistrum*) was the predominate weed species, but cutleaf evening primrose (*Oenothera laciniata*) and swinecress (*Coronopus* sp.) were also present. Over time, wild radish became the dominate species and suppressed organic onion growth. Only hand weeding allowed for the onion to grow season long without competition. The combination of cultivation and hand weeding were effective enough to control weeds as compared to hand weeding alone. Tine weeder did not provide effective control when used twice or four times early in the season. Overall the objective of this research was achieved as tine weeding did reduce the number of weeds and when used in combination with hand weeding, the overall productivity of organic onion could be achieved. However, further research is required to establish how further tine weeding could be used to mitigate other weed species. Monitoring thrips and natural enemies, and predators: Monitoring of thrips, predators and natural enemies were conducted in the same field trial where weed control options were evaluated. Sampling of field plots were conducted three times after the final weed cultivation on the following dates: March 09, 2020, March 16, 2020, and March 23, 2020. One sticky card and one pitfall was placed in the center of each plot and collected after 7 days to account for aerial and ground-dwelling arthropod activity. On March 25, 2020, plots were examined for *Pantoea* sp. severity. Onion yield data was collected on May 14, 2020 and data for the five primary weed species observed was taken on multiple dates over the course of the field trial. Further statistical analysis needs to be conducted, however initial analysis suggests a negative correlation between disease severity and yield per bulb; as *Pantoea* sp. severity increases, yield decreases. On the other hand, wild radish biomass has a positive correlation to foliar disease severity, suggesting disease severity increases with reduced weed management. In addition, yield and wild radish biomass have a negative correlation, where yield decreases as weed abundance increases. Sticky cards are currently being examined for arthropod identification, including thrips abundance. A total of 72 pitfalls and 72 sticky cards were collected. All arthropods in the pitfall traps were recorded to family or lowest taxonomy to our capability. Lower classification may be done if deemed necessary. A total of 1890 arthropods were collected, with the predominant ground-dwelling predators being Araneae and Carabidae. Initial analysis shows relatively higher natural enemy abundance in the non-treated plots and lower abundance of arthropods in the tine weeded 4x with hand-weeding plots. For objective 3, integration of management tactics will be conducted in year 2 and 3 of the project period. PI Dutta gave updates on onion bacterial disease management options in 5 different county meetings in Georgia, January-March 2020 (n=314 attendees in total). For objective 4, Stakeholder advisory panel will meet in November 2020 (virtually) where PD and Co-PIs will share the results of year 1 trial and receive inputs.

****PUBLICATIONS (not previously reported):**** 2019/09 TO 2020/08 1. Type: Journal Articles Status: Accepted Year Published: 2020 Citation: Stumpf, S., Leach, L., Srinivasan, R., Coolong, T., Gitaitis, R., and Dutta, B. 2020. Foliar chemical protection against *Pantoea ananatis* in onion is negated by thrips feeding. *Phytopathology* 2. Type: Journal Articles Status: Under Review Year Published: 2020 Citation: Zhao, M., Kvitko, B.H., Gitaitis, R.D., and Dutta, B. 2020. Bacterial streak and bulb rot of onion. *Plant Health Instructor* (under review) 3. Type: Other Status: Published Year Published: 2020 Citation: Dutta, B., and Gitaitis, R. 2020. Bacterial Diseases of onion in Georgia. *University of Georgia Cooperative Extension Bulletin B1534*. 4. Type: Other Status: Published Year Published: 2020 Citation: Onion World July/August 2020 issue has a short Disease Quiz  submitted by Bhabesh Dutta and Ron Gitaitis to highlight onion bacterial diseases. <https://onionworld.net/magazine/> 5. Type: Other Status: Published Year Published: 2019 Citation: Arboleda, K. 2019. UGA Researchers working on disease control in *Vidalia* onions. *Vegetable and Specialty Crop News*, December 2019. 6. Type: Other Status: Published Year Published: 2019 Citation: Thompson, C. 2019. Center rot disease in organic onion growing studied. *Vegetable Growers News*, November 2019. 7. Type: Conference Papers and Presentations Status: Other Year Published: 2020 Citation: Dutta, Bhabesh (2020). Bacterial Diseases of Onion. Invited presentation at the Annual Meeting of the National Onion Association, August 11, 2020 (virtual meeting).

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Balancing Soil Nutrition for Sustainable Weed and Pest-insect Management

Accession No.	1020496
Project No.	GEOW-2019-03516
Agency	NIFA GEOW
Project Type	OTHER GRANTS
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Contract / Grant No.	2019-51106-30188
Proposal No.	2019-03516
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NON-TECHNICAL SUMMARY

Manure fertilizers bring many benefits for plant and soil health. However, because manure is not composed with an ideal balance of macro/micronutrients for crops, meeting the needs for nitrogen can lead to accumulations of surplus phosphorous. Nutrient imbalances, in turn, may exacerbate weed and insect pest pressure, bringing unexpected and unwelcome management costs to growers. We propose three research objectives to help transitioning organic farmers use fertility inputs to achieve balanced soil nutrition that maximizes profitability and optimizes pest management, focusing on zucchini crops (*Cucurbita pepo* subsp. *pepo*), due to their economic importance and challenging weed and insect problems. First, we will search for links between soil nitrogen, phosphorous, and densities of weeds and pests, on a network of more than 50 collaborating organic mixed-vegetable farms across the Southeast. Second, we will experimentally define how different manure compost application rates impact interactions between weeds, insects, and crop yield. Third, we will optimize combinations of cover crops and different compost fertilizers that improve nutrient balance while promoting natural pest suppression. Complementary economic analyses will allow growers to confidently ensure profitability while minimizing labor investments, while on-farm work with our large network of cooperating growers will ensure real-world applicability of our findings while facilitating outreach efforts. We will extend our findings to a broader audience through workshops, field days, and electronic media. Altogether, our project addresses ORG priorities by quantifying costs and benefits of organic manure composts for biodiversity, soil health, and natural pest control.

OBJECTIVES

We propose three complementary research objectives. 1. Search for links between soil N/ P levels and densities of weeds and pests, on a network of more than 50 collaborating, southeastern organic mixed-vegetable farms. By establishing an interactive network of collaborating farmers at this scale, we gather real-world knowledge, extend it through individualized engagement with farmers, and build natural outreach channels. 2. Experimentally define how different manure compost application rates impact interactions between weeds, insects, and crop yield for organic zucchini crops. This will help us quantify time/money farmers invest in extra weed and pest management because of nutrient imbalances. Economic analyses of labor inputs under different fertility strategies will be

shared with farmers during field days and through eOrganic videos.3. Optimize combinations of cover crops and blends of manure compost (high P) and food waste compost (lower P) to ease P buildup and limit pest susceptibility. We will test some potential solutions for the fertility and pest management challenges we define in Objective 2, alongside economic analyses comparing the relative merits of different fertility materials. In turn, these results will inform our field days and online videos.

APPROACH

Objective 1: Search for links between soil nitrogen, phosphorous, and densities of weeds and pest insects on a network of 50 collaborating organic vegetable farms across the Southeast. With our first objective, we will measure numerous plant-based variables (cover crop and weed diversity) and soil-based variables (micro and macronutrients, organic matter, microbial respiration, etc.) on working organic farms. We will gather this information through a massive on-farm survey project, supported by over 50 mixed-vegetable, organic farmers. Our survey teams will radiate from three locations in different regions of the Southeast with distinct soil and environmental characteristics: Charleston, Greensboro/Charlotte, and Athens/Atlanta. Many of our cooperating growers have integrated crop-livestock operations and incorporate animal-based fertility amendments into their soils; therefore, we expect to observe strong variation in soil microbial communities and invertebrate communities across sites. If we can detect broad patterns that persist among the significant ecological variation in our three survey regions, we can be confident that our results might have broader implications for many farmers throughout the region and country.

Objective 2: Experimentally define how manure compost application rates impact interactions between weeds, insects and crop yield for organic zucchini crops. In this experiment, we will manipulate application rates of composted poultry manure at four levels to determine the point at which excess fertility begins to threaten profitability due to increased pest pressure. We focus on composted poultry manure because it is the most common organic fertility resource used in the Southeast. The five fertility treatments will be arranged in 6 x 8m plots in a latin square design, with 5 replicates (25 plots total). Plots will have five rows each, with 24 squash plants (*C. pepo* subsp *pepo* cvs. 'Yellow fin') per row, and data will be collected from the three interior rows. Weeds will be controlled with scuffle hoes bi-weekly (transitioning farmers often have limited access to expensive implements), and labor investments for each treatment will be estimated for economic analyses.

Objective 3: Optimize combinations of cover crops and blends of manure compost (high P) and food waste compost (low P) to ease P buildup and limit pest susceptibility. Blending manure fertilizers with lower-P alternatives might improve nutrient balance and minimize pest pressure. To examine the role of surplus P in pest susceptibility for manure-fertilized crops, we will manipulate blends and concentrations of two commercially available organic fertility composts that vary in their N:P ratios. Poultry manure compost has a relative low N:P ratio (approximately 1.25) and more surplus P while food/yard waste compost has a relatively high N:P ratio (approximately 1.8), and more balanced P concentrations. Our experiment will be executed with 10 treatments over an ordinal gradient of P, holding total N constant (Plant-available N + potential mineralizable N). We will engineer this P gradient by varying the proportion of N coming from each fertility resource (poultry manure compost and food waste compost) in a substitutive design over 10 levels (Table 1), with four replicates of each level in randomized blocks (40 total 6 x 8m plots with five rows). Treatments with high proportions of N coming from poultry manure (e.g. Symphony® 5-4-3) will have relatively high concentrations of P, whereas treatments with high concentrations of food waste compost (e.g. Atlas Organics®, 1.1, 0.6, 0.39) will have relatively lower P. We have selected two fertility materials with similar concentrations of organic matter, but because we focus on commonly applied, commercially available, and agriculturally relevant organic fertilizers, other micro and macronutrients will vary. We will apply our gradient of N:P ratios in combination with the most common fall cover cropping strategy in our area: a fall cereal rye/clover cover crop which will be mowed and integrated in the spring in each plot ahead of our experiment. This approach will enable us to identify pest suppressive blends of manure and other organic fertility resources (cover crops and food waste compost) with lower P concentrations.

Progress 09/01/19 to 08/31/24

Outputs Target Audience: The goal of our ORG project was to help farmers use fertility inputs to achieve balanced soil nutrition that maximizes profitability and optimizes pest management, focusing on zucchini crops (*Cucurbita pepo* subsp. *pepo*) due to their economic importance and challenging weed and insect problems. Our target audience was organic vegetable growers who rely on organic inputs to improve soil fertility, but often struggle to achieve soil nitrogen targets without adding too much phosphorous. In addition, we hope that our work provided basic insight into how soil nitrogen and phosphorous in the soil indirectly impact plants' ability to defend themselves against herbivorous insects, and compete with other plant species.

Changes/Problems: Nothing Reported

What opportunities for training and professional development has the project provided? Three graduate students (Julia Berliner, Amy Sparer, and Carly Sharp), as well as undergraduate research assistants (Melina Madden, Andrew Godard, and Logan Johnson) were trained or were trained on this project. Melina and Andrew led efforts to quantify how weed pressure and weed management labor were influenced by surplus fertility applications, and Melina and Amy led on-farm surveys of soils and pests across South Carolina. Julia led a second year of a field experiment (Obj 2) in 2021, and Carly conducted a series of field experiments in the

2022-2024 growing seasons. How have the results been disseminated to communities of interest? Results were presented at a series of Entomological Society of America meetings, and in an annual webinars with our network of participating organic farmers. Youtube videos on identifying common pests and predators in cucurbit systems were composed. We also prepared display boxes of common pests and natural enemies in cucurbit production, and these have been presented during on-farm surveys and greatly appreciated by farmers who were previously unable to develop search images for common beneficial insects (many of whom are rather small and difficult to identify). We have engaged grower-collaborators on this project and have had on-farm discussions with most growers to gauge their concerns associated with fertility management. We prepared individualized reports of soil tests and biodiversity inventories of plants and insects for the farmers in our network. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported Impacts What was accomplished under these goals? Objective 1: Work with a network of over 50 organic mixed-vegetable farms in the Southeast to search for links between soil nitrogen, phosphorous, and densities of weeds and pests. We visited more than 50 organic farms across Georgia and the Carolinas, and collected soil samples, evaluated weed communities in quadrat surveys, performed visual surveys and vacuum samples of pests and natural enemies, and performed sentinel prey assays. We grew zucchini plants in soils collected from each farm in a common garden experiment, and measured rates of herbivore growth and pest suppression by natural enemies. We found that while herbivore growth did not correlate with increased soil N (as we predicted), omnivorous predators linked more tightly with soil N and plant quality, potentially counterbalancing effects of surplus fertility on pest growth. This research was published in the Journal of Animal Ecology. Objective 2: Experimentally define how different manure compost application rates impact interactions between weeds, insects, and crop yield. We completed a two-year field experiment manipulating concentrations of poultry manure fertilizers on zucchini plants. We predicted that surplus fertility conditions would exacerbate pest pressure, but found instead that pests had neutral or inconsistent responses to fertility, while natural enemies increased under surplus fertility conditions. These results were incorporated into project students Julia Berliner's MS thesis (defended fall 2022). A second manuscript evaluating the consequences of surplus fertility for weeds, pests, plant pathogens, and profitability is in preparation. Objective 3: Optimize combinations of cover crops and different compost fertilizers that improve nutrient balance while promoting natural pest suppression. MS student Janeva Williams & Co-PD Bhowmik (NC A&T) examined impacts of cover cropping and manure application on greenhouse gas emissions and impact of biochar application on soil enzymes as biological indicators of soil health. Janeva and Co-PD Bhowmik presented their research at seven conferences and workshops in 2021-2022, and defended her MS thesis in 2022. Co-PD Bhowmik also published a review paper on organic amendments that improve soil health in *Frontiers in Microbiology*. PhD student Carly Rae Sharp led an experiment manipulating ratios of nitrogen and phosphorus in organic fertility treatments on zucchini crops, and found that high concentrations of phosphorus (common in manure-fertilized systems) increased weed growth, while limiting crop growth and reducing attraction by beneficial insects, which indirectly increased pest pressure. Carly's research was presented at the Southeastern Branch meeting of the Entomological Society of America in 2023, and her work will be submitted for publication in 2025. Publications Type: Peer Reviewed Journal Articles Status: Published Year Published: 2023 Citation: Blubaugh, CK. An omnivore vigour hypothesis? Nutrient availability strengthens herbivore suppression by omnivores across 48 field sites. *Journal of Animal Ecology* 92:751-759. Progress 09/01/22 to 08/31/23 Outputs Target Audience: The goal of our ORG project is to help farmers use fertility inputs to achieve balanced soil nutrition that maximizes profitability and optimizes pest management, focusing on zucchini crops (*Cucurbita pepo* subsp. *pepo*) due to their economic importance and challenging weed and insect problems. Our target audience is organic vegetable growers who rely on organic inputs to improve soil fertility, but often struggle to achieve soil nitrogen targets without adding too much phosphorous. In addition, we hope that our work will provide basic insight into how soil nitrogen and phosphorous in the soil indirectly impact plants' ability to defend themselves against herbivorous insects, and compete with other plant species. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? This project supported MS student Julia Berliner, and PhD student Carly Sharp. How have the results been disseminated to communities of interest? Nothing Reported What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported Impacts What was accomplished under these goals? Objective 1: Work with a network of over 50 collaborating organic mixed-vegetable farms in the Southeast to search for links between soil nitrogen, phosphorous, and densities of weeds and pests. We visited more than 40 organic farms across Georgia and the Carolinas, and collected soil samples, evaluated weed communities in quadrat surveys, performed visual surveys and vacuum samples of pests and natural enemies, and performed sentinel prey assays. In 2021, we grew zucchini plants in soils collected from each farm in a common garden experiment, and measured rates of herbivore growth and pest suppression by natural enemies. We analyzed these data with the on-farm samples, and found that while herbivore growth did not correlate with increased soil N (as we predicted), yet omnivorous predators linked more tightly with soil N and plant quality. These results have been analyzed. Objective 2: Experimentally define how different manure compost application rates impact interactions between weeds, insects, and crop yield. MS student Julia Berliner repeated a field experiment manipulating concentrations of

poultry manure fertilizers on zucchini plants. We predicted that surplus fertility conditions would exacerbate pest pressure, but they found that pests (mostly dominated by specialist squash bugs) actually declined under high fertility conditions, while natural enemies increased under surplus fertility conditions. These results have been analyzed. Objective 3: Optimize combinations of cover crops and different compost fertilizers that improve nutrient balance while promoting natural pest suppression. PhD student Carly Rae Sharp has been recruited and has begun leading field experiments to address this goal. Publications Progress 09/01/21 to 08/31/22 Outputs Target Audience: The goal of our ORG project is to help farmers use fertility inputs to achieve balanced soil nutrition that maximizes profitability and optimizes pest management, focusing on zucchini crops (*Cucurbita pepo* subsp. *pepo*) due to their economic importance and challenging weed and insect problems. Our target audience is organic vegetable growers who rely on organic inputs to improve soil fertility, but often struggle to achieve soil nitrogen targets without adding too much phosphorous. In addition, we hope that our work will provide basic insight into how soil nitrogen and phosphorous in the soil indirectly impact plants' ability to defend themselves against herbivorous insects, and compete with other plant species. Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? Three graduate students (Julia Berliner, Amy Sparer, and Carly Sharp), as well as undergraduate research assistants (Melina Madden, Andrew Godard, and Logan Johnson) were trained on this project. Melina and Andrew led efforts to quantify how weed pressure and weed management labor were influenced by surplus fertility applications, and Melina and Amy led on-farm surveys of soils and pests across South Carolina. Julia led a second year of a field experiment (Obj 2), and Carly conducted a series of field experiments. How have the results been disseminated to communities of interest? Pest/predator display collections: in 2021, UGA students (led by Co-PD Blubaugh) curated six small display collections featuring the most common pests and natural enemies on organic farms for use in informal farmer training during on-farm visits and at outreach events. Pest/predator videos: In 2021 and 2022, UGA students (led by Co-PD Blubaugh) created 13 short, engaging videos featuring common pests and beneficial insects to aid farmers in identification. (link to YouTube playlist) What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported Impacts What was accomplished under these goals? Objective 1: Work with a network of over 50 organic mixed-vegetable farms in the Southeast to search for links between soil nitrogen, phosphorous, and densities of weeds and pests. We visited more than 50 organic farms across Georgia and the Carolinas, and collected soil samples, evaluated weed communities in quadrat surveys, performed visual surveys and vacuum samples of pests and natural enemies, and performed sentinel prey assays. In 2021, we grew zucchini plants in soils collected from each farm in a common garden experiment, and measured rates of herbivore growth and pest suppression by natural enemies. We found that while herbivore growth did not correlate with increased soil N (as we predicted), omnivorous predators linked more tightly with soil N and plant quality, potentially counterbalancing effects of surplus fertility on pest growth. Objective 2: Experimentally define how different manure compost application rates impact interactions between weeds, insects, and crop yield. MS student Julia Berliner and undergraduate Mary-Frances Behnke completed a two-year field experiment manipulating concentrations of poultry manure fertilizers on zucchini plants (mentored by Co-PD Blubaugh). We predicted that surplus fertility conditions would exacerbate pest pressure, but found instead that pests had neutral or inconsistent responses to fertility, while natural enemies increased under surplus fertility conditions. These results were incorporated in Julia's MS thesis (defended fall 2022) and are currently in-review at Ecology. A second manuscript evaluating the consequences of surplus fertility for weeds, pests, plant pathogens, and profitability is in preparation (Led by Blubaugh, with Cutulle and Vassallos). Berliner, J.T., Behnke, M.F., Hagan, K.L., and Blubaugh, C.K. Diet breadth mediates arthropod responses to nutrient availability across trophic levels. (In review at Ecology) Objective 3: Optimize combinations of cover crops and different compost fertilizers that improve nutrient balance while promoting natural pest suppression. During 2021 and 2022, MS student Janeva Williams & Co-PD Bhowmik (NC A&T) examined impacts of cover cropping and manure application on greenhouse gas emissions and impact of biochar application on soil enzymes as biological indicators of soil health. Janeva and Co-PD Bhowmik presented their research at seven conferences and workshops in 2021-2022, and defended her MS thesis in 2022. Co-PD Bhowmik also published a review paper on organic amendments that improve soil health in *Frontiers in Microbiology*. During summer 2022, MS student Carly Rae Sharp & PD Snyder (UGA) led an experiment manipulating ratios of nitrogen and phosphorus in organic fertility treatments on zucchini crops, and found that high concentrations of phosphorus (common in manure-fertilized systems) increased weed growth, while limiting crop growth and reducing attraction by beneficial insects, which indirectly increased pest pressure. Carly's research was presented at the Southeastern Branch meeting of the Entomological Society of America. Publications

PROGRESS

2020/09 TO 2021/08 Target Audience: The goal of our ORG project is to help farmers use fertility inputs to achieve balanced soil nutrition that maximizes profitability and optimizes pest management, focusing on zucchini crops (*Cucurbita pepo* subsp. *pepo*) due to their economic importance and challenging weed and insect

problems. Our target audience is organic vegetable growers who rely on organic inputs to improve soil fertility, but often struggle to achieve soil nitrogen targets without adding too much phosphorous. In addition, we hope that our work will provide basic insight into how soil nitrogen and phosphorous in the soil indirectly impact plants' ability to defend themselves against herbivorous insects, and compete with other plant species.

Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? Three graduate students (Julia Berliner, Amy Sparer, and Carly Sharp), as well as undergraduate research assistants (Melina Madden, Andrew Godard, and Logan Johnson) are currently being trained or were trained on this project. Melina and Andrew led efforts to quantify how weed pressure and weed management labor are influenced by surplus fertility applications, and Melina and Amy led on-farm surveys of soils and pests across South Carolina. Julia led a second year of a field experiment (Obj 2) in 2021, and Carly will lead one for objective 3 in 2022. How have the results been disseminated to communities of interest? Preliminary results were presented at the Entomological Society of America meeting virtually, and in an annual webinar with our network of participating organic farmers. Youtube videos on identifying common pests and predators in cucurbit systems have been composed will be published in 2021. We also prepared display boxes of common pests and natural enemies in cucurbit production, and these have been presented during on-farm surveys and greatly appreciated by farmers who were previously unable to develop search images for common beneficial insects (many of whom are rather small and difficult to identify. We have engaged grower-collaborators on this project and have had on-farm discussions with most growers to gauge their concerns associated with fertility management. Late in 2021, we prepared individualized reports of soil tests and biodiversity inventories of plants and insects for the farmers in our network. What do you plan to do during the next reporting period to accomplish the goals? Objective 1) This objective is technically complete, but we plan to continue on-farm sampling in the long-term to maintain ongoing relationships with farmers in our survey network. Objective 2) MS student Julia Berliner draft a manuscript examining how surplus macronutrients influence weeds, insect pests, and biological control services by natural enemies. These results will be communicated to growers in an annual webinar (February 2022), and at the Entomological Society of America Conference. Objective 3) MS student Carly Sharp will lead an experiment manipulating a gradient of N:P ratios in fertilizers by blending poultry manure (high P) with feathermeal-based fertilizers (low P) in cover cropped environments to reveal optimal cocktails of common fertility amendment materials that support crop productivity while minimizing pest susceptibility.

IMPACT

2020/09 TO 2021/08 What was accomplished under these goals? Objective 1: Work with a network of over 50 collaborating organic mixed-vegetable farms in the Southeast to search for links between soil nitrogen, phosphorous, and densities of weeds and pests. In 2021, we visited more than 40 organic farms across Georgia and the Carolinas, and collected soil samples, evaluated weed communities in quadrat surveys, performed visual surveys and vacuum samples of pests and natural enemies, and performed sentinel prey assays. In 2021, we grew zucchini plants in soils collected from each farm in a common garden experiment, and measured rates of herbivore growth and pest suppression by natural enemies. We analyzed these data with the 2020 on-farm samples, and found that while herbivore growth did not correlate with increased soil N (as we predicted), yet omnivorous predators linked more tightly with soil N and plant quality. These results have been analyzed, and a paper was submitted to Ecology in 2021. Objective 2: Experimentally define how different manure compost application rates impact interactions between weeds, insects, and crop yield. MS student Julia Berliner repeated a field experiment manipulating concentrations of poultry manure fertilizers on zucchini plants. We predicted that surplus fertility conditions would exacerbate pest pressure, but they found that pests (mostly dominated by specialist squash bugs) actually declined under high fertility conditions, while natural enemies increased under surplus fertility conditions. These results have been analyzed and presented at the Entomological Society of America meeting, and will be prepared for submission in 2021. Objective 3: Optimize combinations of cover crops and different compost fertilizers that improve nutrient balance while promoting natural pest suppression. MS student Carly Rae Sharp has been recruited to lead this experiment in the 2022 field season. **PUBLICATIONS (not previously reported):** 2020/09 TO 2021/08 No publications reported this period.

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Transitioning to Organic Grain Production: Strategies to Maximize Profitability and Ecosystem Services While Reducing Risks and Barriers

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NON-TECHNICAL SUMMARY

Farming in the Delmarva (Delaware-Maryland-Virginia) region is dominated by commodity grain production (particularly corn, soybeans, and wheat) which has inherently low value (~\$600) and profit (~\$200) per acre, resulting in a trend toward fewer but larger farms. Organic grain production has the potential to boost farmer profits via premium prices about double conventional prices and new markets opening up for organic corn and soybean. Thus converting to organic grain production has the potential to greatly enhance farmer income, even if organic yields are substantially lower than conventional. However, the three-year transition period required to achieve organic farming status presents a major barrier to wider organic grain production because during this period farmers receive only conventional prices but must forgo their customary use of synthetic fertilizers, herbicides, and pesticides upon which they have depended for high-yield production. As a result, farmers are faced with producing significantly lower yields, sometimes at higher costs, while receiving conventional prices for their grain. These factors result in the likelihood of farmers experiencing much lower profits, or even net losses, during the three transition years. The US as a nation is the world's major exporter of corn, soybeans and other grains, while it has had to import most of its organically certified supply of these feed grains to meet the demands of the US organic poultry and livestock industries. The demand and prices for organic grain are so much higher than for conventional grain that fraud is being uncovered. To meet the growing demand, the amount of certified land producing organic corn and soybeans will have to triple from the current 650,000 acres to approximately 2 million acres in the next few years. Chicken producers in the region are aggressively seeking domestic sources of organic grain to lower their dependence on imports. Further, low milk prices are spurring dairy farmers toward organic which will require still more organic grains. Thus, there is a great need to find ways to make the transition to organic production easier and less risky for grain farmers. Consumers purchase organic food both for food safety (fewer pesticide residues) and for environmental reasons (less impact on water quality and climate). There is good evidence that organic foods do, indeed, contain lower levels of pesticide residues than conventionally produced food, although this difference is less for grain crops than for fruits and vegetables. Organic farming is often perceived as also having fewer damaging impacts on the environment compared to other farming systems that use synthetic chemicals. However, the change in environmental impacts due to the transition from "conventional" to organic farming could be either positive or negative, depending on the particular system of practices utilized in the conventional and organic systems. In Maryland, most grain farms considered to be "conventional" practice some form of no-till agriculture, grow some cover crops and use strict nutrient management plans with split fertilizer applications and restricted application rates of animal manures, nitrogen,

and phosphorus fertilizers. Most organic farmers use animal manures as well as legume cover crops to maintain soil fertility and rely on tillage to prepare seedbeds, incorporate amendments, and control weeds. Since the ratio of available P/N in manures and composts far exceeds that required by crops, manure use is restricted by Maryland nutrient management regulations to allowable P levels, often restricting or even excluding manure and compost as fertility options for organic farmers. These limitations on manure and compost and the very high cost of organic fertilizers that are high in N but low in P make cover crops especially important to organic farms. Whether converting land from conventional to organic grain production would reduce or increase environmental impacts is an open question, especially in the Delmarva region where the majority of conventional farmers already employ such conservation practices as integrated pest management, nutrient management, and cover crops. Since Maryland grain farmers predominantly use no-till or minimum-till soil management, a switch to traditional organic farming methods could involve significant increases soil disturbance, likely accompanied by greater erosion, and runoff losses, especially during the transition period when soil organic matter has not yet been built up. Also, organic and vegetative (legume) nitrogen inputs are not as easily and precisely timed and controlled as are inorganic fertilizer, so it is unclear as to whether nutrient losses would decrease or increase with conversion to organic farming. Organic production, per se, may not reduce environmental impacts such as sediment and nutrient loss and greenhouse gas emissions. However, organic grain production, if done regeneratively, may minimize these environmental impacts and provide substantial ecosystem services. Among these, nutrient loss reduction is vitally important given that about 40% of nutrient pollution to the Chesapeake Bay comes from agriculture. Other ecosystem services from regenerative organic farming may include carbon sequestration, soil erosion control, protection of above and below-ground biodiversity, and provision of clean water to aquifers and streams. This project aims to research a suite of practices that make organic farming more regenerative than typically practiced. Therefore, the adoption of organic farming presents a rare opportunity for grain farmers, especially those on the Delmarva Peninsula, to realize increased income as well as potentially provide better stewardship of their land and water. This project's overall aim is to develop strategies and practices to minimize soil disturbance, environmental impacts, and input costs while providing profits during the transition comparable to those realized under conventional grain farming. We propose an integrated research-extension project to mentor transitioning farmers and develop systems to minimize soil disturbance, environmental impacts, and input costs while providing profitability during the transition period. The research goal is to compare four transition strategies along a continuum of soil disturbance, soil cover, and input cost. These will be replicated in farm-equipment scale plots on contrasting soil at two research stations and on two or more commercial farms. They will be evaluated for effects on soil health, leaching and runoff nutrient loss, crop yields, profitability, and ease of management. In order of least to most disturbance, the four systems are 1) Perennial alfalfa-grass hay, untilled; 2) Minimum-till corn-soybean-wheat rotation with precision-zoned high-biomass diverse cover crops; 3) Reduced-till corn-soybean-wheat with high-biomass cover crops; and 4) Traditional full-tillage organic soybean-corn, with simple cover crops and input substitution (using organic-approved forms of the same types of inputs used by conventional farms). The project will assess the impacts of these transition strategies on soil health, farm profitability, and crop productivity. It will include an outreach component that involves farmers in the research and employs experienced organic grain farmers in mentoring farmers who are interested in learning how to transition to organic production.

OBJECTIVES

Project objectives: This project directly addresses priority areas 1, 2, and 4 of the USDA Organic Transitions RFP. Furthermore, our project will directly address at least two areas of research recommended as priorities by the National Organic Board (National Organic Standards Board, 2018), namely "#2. Organic no-till practices for diverse climates, crops, and soil types" and "#8. Production and yield barriers to transitioning to organic production to help growers successfully complete the transition." University of Maryland, UME, and FH-CASA will build on their long-standing partnership to work together on a suite of fully integrated research and extension activities to encourage and better support farmers making the transition to organic production. Our overall goal for this project is to demonstrate and compare the feasibility and relative advantages and disadvantages of four alternative cropping systems for the transition to organic grain production. We hypothesize that systems with less disturbance and less reliance on purchased inputs will also produce less N and P losses to the environment.

Research objectives: This project's research component's overall aim is to study four different transitional cropping strategies/rotations that represent a continuum of soil disturbance, soil cover, and input intensity. They will be evaluated for effects on soil health characteristics, leaching water quality, crop yields and profitability, and ease of management. This interdisciplinary project aims to improve the profitable management of crops and soils in transitional and organic farming. It will address knowledge gaps regarding effects of minimal- and low-tillage transition systems on soil physical, chemical, and biological properties (see Table 2), as well as on profitability, nutrient leaching losses, and the learning curve for farmers used to using no-till soil management and herbicide-terminated cover crops. The specific objectives of the research, which were developed in response to

interdisciplinary stakeholder input, are to 1) provide farmers with practical, field-tested information about the agronomic and economic performance and management challenges of four different approaches to transition to organic grain production; 2) determine how the four transition systems affect soil health and 3) contribute to a growing scientific body of knowledge on profitability and ecosystem services (including carbon sequestration, soil organic matter accumulation or loss, and water quality) provided by a continuum of transitional strategies in a range of environments. The research component - via its on-farm and on-station trials and geographic focus on Maryland's Delmarva Peninsula - is designed specifically to be transferable to farmers and service providers operating in, and scientists studying, grain production systems in the Mid-Atlantic and, with appropriate consideration of climate and soils, in other regions, as well. For example, although cover crops are widely used in the Delmarva region, most are minimally managed, consisting of wheat or rye planted late in fall and terminated early in spring with little biomass accumulation and minimal agronomic and environmental benefits. Likewise, most US organic grain growers use full tillage (leading to soil erosion and reduced soil health) and high amounts of manure and compost (that can lead to excessive P levels and losses). While there has been some recent research (Finney et al. 2017) on environmental benefits of precision-zoned, high-biomass, diverse cover crops, such cover crops are rare in the Delmarva region. The current study seeks to fine-tune the management of and demonstrate environmental and economic incentives for using such cover crops, while at the same time providing the logistical support and mentorship necessary for farmers to be willing to adopt these and other soil health-promoting practices in organic farming. Extension objectives: The objectives of the project's extension components, based on the interdisciplinary stakeholder input above, are to 1) extend the project's research findings to grain farmers, agriculture educators, and service providers so they can make informed decisions when tackling, or helping others to tackle, the transition period; 2) extend the project's research on ecosystem services provided by the researched transition strategies to other scientists and farmers and stakeholders, including to the Chesapeake Bay Program, which is ramping up efforts to address nonpoint sources of pollution; and 3) provide support and mentorship for grain farmers with the further goal of increasing the number of organic grain producers by a minimum of 15. All three objectives will focus on increasing farmer understanding of benefits of certification; reducing barriers to certification, and offering support and mentorship during the transition and the first two years under certification.

APPROACH

Project staff will assemble an advisory committee of stakeholders to help to lead the direction of the educational program and assist with outreach and marketing, ensuring that the project continues to meet real-world needs and that all potential transitional farmers and their service providers are aware of the project's programs and research results. Research Activities: Four systems representing a continuum of tillage intensity, cover crop optimization, and purchased inputs will be compared during the three-year transition period are (starting with August/September of Year 1). In addition, the initial certified organic corn crop will be assessed. Crops harvested for sale are in bold: - Alfalfa-grass \>alfalfa-grass hay \>alfalfa-grass hay \>corn. All N from legumes. 1 tillage in 3 yrs. - Radish-oat-Sunn hemp cover crop\>corn\>rye cover crop\>soybean\>wheat\>radish-oat-Sunn hemp cover crop\>corn. Most N from cover crops, legumes, some poultry manure. Minimum tillage. - Vetch-rye cover crop\>corn\>rye cover crop\>soybean\>wheat\>vetch-rye cover crop\> corn. Most N from cover crops, legumes, some from poultry manure. Reduced tillage. - Rye cover crop\>soybean\>vetch-rye cover crop\>corn\>rye cover crop\>soybean\>vetch-rye cover crop\>corn. Most N from purchased poultry manure & compost or org. fertilizers. Full tillage. Experimental Design: These four systems will be compared in replicated trials both on experiment station land and on commercial farms. All four systems will be compared in randomized complete block experiments on two contrasting soils representing the coarser and finer textured soils typical of the Delmarva's grain cropping coastal plain region. In addition, field-scale plots will be established on two or more private farms on the Delmarva Peninsula, comparing between three and four of the experimental transition strategies, according to the interests and facilities of the farmers in question. Three or four system treatments will be replicated on each farm at least 3 times, with either whole fields or large strip plots serving as the experimental units. When fields are used as experimental units, they will be matched as far as possible to have similar soil type and management history. Within each experimental unit, representative and relatively uniform sampling plots approximately 45 ft (13.7m) wide and 150 ft (35.7m) long (sizes depends on equipment and field geometry) will be established by field markers and GPS to provide for repeated soil, leachate water, and plant sampling. Response variables (costs, returns, profitability, yields, soil properties, and leaching and runoff losses) will be analyzed statistically with R and SYSTAT packages using General Linear Models and regression analysis. When comparable data are available for a suite of two or more Systems, the analyses will be combined across on-station and on-farm sites. Indices of disturbance, input, and labor intensity will be calculated for each system. Multivariate analyses will be conducted to compare the systems with regard to the whole suite of soil, economic and agronomic variables. In addition to the systems experiments, it may be necessary to conduct a few much smaller, single-factor experiments at one or more sites to answer specific or mechanistic questions that arise.

Analytical Methods: A suite of detailed data will be collected three times per year: stand establishment, crop yield, crop value, soil health parameters, weed population and biomass, pest infestation (occurrence, population densities, and damages), and seasonal trends for soil temperature and water. Suction lysimeters installed to 1 m depth in both research station and on-farm plots (three per plot) will be sampled during the fall-winter-spring leaching season and the water analyzed for nitrate, total dissolved N, C and P (using Lachat and Shimadzu instruments). Soil Chemical and Physical Properties. Soil bulk density, porosity, and the water stability of 4 mm diameter aggregates will be determined from large diameter (7.5 cm) cores taken from the surface 7.5 cm soil each spring. At ten locations per plot within a meter of the above coring, soil strength (penetration resistance) will be measured simultaneously with volumetric soil water content using a recording penetrometer and capacitance probe. This will allow correction of soil strength measurements for soil water content effects. Temperature and moisture of the surface soil (7.5 cm) will be monitored using a combined capacitance water content sensor and thermistors in early spring near planting time and again in summer during periods of potential heat and drought stress. Soil Quality: Biological Properties. Arthropod and Earthworm Community Response. Soil samples for arthropod and earthworm assays will be collected three times annually: September, April-May, and June-July. For each sampling occasion, five randomly located undisturbed soil cores 10-cm deep x 7.5 cm diameter will be collected per plot. Diversity indices will be used to determine treatment effects on soil micro-arthropods diversity. Earthworms will be enumerated using a mustard extrudition method, backed up by counting middens on the soil surface and excavation and hand sorting to 20 cm depth. Slugs will be assessed using shingle traps in spring and plant damage to young crops. Carbon and nitrogen lability will be assessed using short term release upon wetting. Active or easily oxidizable soil carbon will be measured as POXC. Soil Health Indices. Because of the complex variables involved, a soil quality index will be developed to simplify the tracking and comparison of changes in soil health. Several soil quality functions will be chosen (such as the provision of water to crops, the resistance of structural degradation, the release of plant-available nutrients, and suppression of plant diseases, etc.) and corresponding indicator properties will be assigned functional scores. A second approach will be more statistical in nature and aims to provide an index that can be used to compare overall soil quality. The t-scores for each variable will be weighted as in the first approach and then averaged to give a relative soil quality index. Economic Analysis: Enterprise budgeting will be conducted for each of the four strategies on both the experiment station land and commercial farm replicated trials. These budgets will track all inputs and outputs during the duration of the study to calculate income, variable costs, and fixed costs for all research plots. These economic data will then be generalized to infer income, expenses, and profit per acre and per unit (hay/ton, grain/bushel) of production. Breakeven for both price and yield will be calculated. This enterprise budgeting analysis will then be generalized to whole-farm budgets to theoretically analyze the long term profitability of the organic crop rotations on commercial-sized farms. The farmers will be required to log their time spent working on each site, crop yield, crop value, and input and fuel expenses. Progress 09/01/19 to 08/31/24 Outputs Target Audience: Scientists researching or interested in organic transitioning or organic farming practices and effects soil health; farmers interested in transitioning or already growing organic crops, along with farm-advisors in private practice or government agencies. Changes/Problems: The project has undergone some major changes as stated in previous annual progress reports. The major changes since the last progress report (during the final year of the project) are: 1) We are continuing to work on peer-reviewed manuscripts and some microbial genomics data for the soil health assessment. 2) One of the four original sites (Farm B) was eliminated from the final cropping year of the project because of excessive wildlife damage by deer and because getting organic certification was made impossible by the farmer inadvertently planting GMO corn as a border around the project area plots to be certified. The yield, soil health, and economic analysis reported for the final year therefore focuses on the other three sites. Several goals that were planned to be achieved by the end of the project in August 2024 are ongoing and will be completed in 2025 without any further funding from the NIFA project. What opportunities for training and professional development has the project provided? One PhD student, Biwek Gairhe, completed his coursework and research and will receive his PhD degree shortly after the project completion date. His dissertation is focused on this NIFA-supported project. Two MS degree students helped work on the project and gained knowledge and experience in organic farming practices even though their dissertations were focused on non-organic research. About 17 undergraduate students gained research experience and knowledge of organic farming practices and challenges by assisting in field and laboratory operations on the project. Of these, seven participated in the laboratory and field work during the final year of the project. Certified crop advisor credits were provided on several occasions by project workshops that educated crop advisors on organic practices and practices for the transition. Approximately 65 crop advisors gained knowledge about organic transition, practices, and barriers to growing organic grain during the Delmarva crop School in Ocean City in November 2023. Farmers and crop advisors learned about organic grain transition practices and challenges during our workshop session at the annual Future Harvest conference in College Park in January 2024 which was attended by mainly farmers interested in or already growing organic crops. How have the results been disseminated to communities of interest? Certified crop advisor credits were provided on several occasions by project workshops that educated crop advisors on organic practices and practices for the transition. A field-day on "Advanced Cover Cropping"

was held on 6 Dec 2023 at three locations in Queen Anne's County, MD. This field day was attended by 82 farmers and farm advisors who learned about organic cover cropping practices at Mason's Heritage organic farm. Approximately 65 crop advisors gained knowledge about organic transition, practices, and barriers to growing organic grain during the Delmarva crop School in Ocean City in November 2023. Farmers and crop advisors learned about organic grain transition practices and challenges during our workshop session at the annual Future Harvest conference in College Park in January 2024 which was attended by mainly farmers interested in or already growing organic crops. An oral presentation to researchers interested in transition to organic grain was made at the international annual meetings of the Agronomy and Soil Science Societies of America in St. Louis, MO in November 2023. Three manuscripts for peer reviewed journals are under preparation. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported

Impacts What was accomplished under these goals? In October 2024 we harvested our first certified organic corn crop across all sites and treatment plots to assess the impacts of 3 years of transitional management strategies. Soil health parameters were measured and we conducted a survey of farmers considering transitioning to organic grain production to ascertain what barriers they faced.

Research objective one: Provide Farmers with practical information about agronomic and economic performance and management challenges.

- 1. Activities.** All the treatment plots were given the same management and planted to corn to compare the residual effects of the previous management on soil quality and productivity. One farm dropped out of organic certification and the program because of deer damage and mistaken use of GMO corn borders. We evaluated the economic performance of treatments at the remaining three sites (CMREC, LESREC, and Farm A) using inputs and outputs to calculate profit per acre.
- 2. Data.** Corn yields were measured by calibrated combine yield monitors and by hand. We also measured crop stand, weed biomass, standard fertility soil tests, and soluble soil nitrate nitrogen at corn V5-7 stage. Forage quality analysis was done on 32 hay samples from CMREC and LESREC (at Dairyland Labs). The economic analysis included costs for materials (seeds, fertilizers, etc) and operations (planting, harvesting, etc), and revenues from crop sales. University of Maryland extension custom operation rates were used. Revenue was calculated using average farmgate prices for the respective years. Profit was calculated as (total revenues) - (total costs) for each crop year and Trt. Maryland cover crops incentives and organic certification costs were omitted. Overall profit was calculated in \$/acre for the 4 year project duration.
- 3. Results.** Due to planter and wildlife problems at LESREC, there was usable yield data at only 2 project sites in 2023. Corn in Trt4 (hay during the transition) far outperformed the other Trts. Yields by hand and combine harvest were correlated but, as expected, hand harvest yields exceeded combine harvest yields, due to less crop loss and avoidance of planting skips or groundhog holes. Hand harvest corn yield at CMREC was ~10,000 kg/ha for Trt4, compared to 4500 to 6500 kg/ha for Trts 1-3. The effects of the Trts on yields varied by site. At CMREC, the sum of corn grain plus weed biomass produced on each plot varied from 9,200 to 12,100 kg per hectare, with no difference between fertilized and unfertilized subplots. Treatments did not affect the sum of corn grain + weed biomass. A regression decision tree that included all soil and plant parameters measured indicated that the soil test K was the first determining factor between the high-yielding and low-yielding plots, probably resulting from potassium sulfate applied only to Trt4 to compensate for the high K removal in harvested hay. The second factor was POXC with low-yielding plots having <243 mgC/kg soil. The final factor was weediness in August with low-yielding plots having >2,400 kg/ha of weed dry matter. Profitability varied across Trts and research sites as influenced by differences in yield and crop selection. At Farm A, Trt1 was most profitable, partly because the farmer was well-acquainted with these practices, but reduced tillage practices were new to him. At CMREC, Trt4 was most profitable, due to excellent 2023 organic corn yields. At LESREC, poor crop stands, wildlife damage, and lack of cultivation equipment caused Trt4 (hay) to be the only profitable Trt. Trt2 (reduced tillage) consistently incurred the lowest costs across sites, but revenue generation was limited. Trt4, despite higher costs, often delivered the greatest profitability. At Farm A, Trt1 produced a total profit of \$1014.91/acre over four years. At CMREC, Trt 4 gave the highest overall profit (\$626.89/acre), despite having to replant the hay crop in year 1. The second highest overall profit (\$365.75/acre over four years) came from Trt1. LESREC suffered net loss or very nominal profit each year in Trts 1-3, primarily due to low crop stand or crop failure. Trt4 was the only treatment with overall profitability (\$520.29/acre) driven by excellent hay crops in 2021 and 2022. Certified organic corn in 2023 was the most consistently profitable crop across all treatments, with profits ranging from \$187.04 to \$755.65/acre.

Research Objective two: determine how the four transition systems affect soil health.

- 1. Activities.** We measured soil respiration, total C and N, dry macro-aggregation, wet macro aggregate stability, penetration resistance with depth, and Mehlich-3 soil tests.
- 2. Data.** To assess the impacts of 3 years of transitional management on soil health parameters at CMREC, LESREC, and Farm A, total C and N were analyzed on July 2023 soil samples (0-15cm and 15-30 cm) and aggregate stability and soil respiration (24 hr and 72 hr CO₂ burst) were analyzed for August 2022 samples (0-10 cm). Soil penetration resistance and infiltration rate were measured at 3 sites. Macroaggregates (1-4 mm) were dry-sieved to get a percent of the whole soil and the stability of those macroaggregates was determined by wet sieving. In addition, soil microbiome analyses are still ongoing.
- 3. Results.** Across 3 sites, the 24-hour CO₂ burst soil (0-10 cm) respiration at 3 sites was significantly higher for the minimum till and no-till (Trs 2 & 3) than for Trt1. This indicates Trts 2 & 3 had accumulated more labile carbon

and active microbial biomass than Trt1. Total soil C and N contents were greater in Trts 3 & 4 than in Trt1, while the C/N ratio was lower in Trt4 than in the other Trts. While there were no treatment effects on the water stability of 1-4mm macroaggregates (all > 95%), the % macroaggregates in the whole soil was greater for the Trts with less tillage (10, 16, 18, and 20% for Trts 1, 2, 3 and 4, respectively). Penetration resistance readings reached a maximum of 2,500 kilopascals at 15 cm and then remained the same below that. Soil penetration resistance increased from 5 to 12.5 cm. At 5, 7.5 and 10 cm, treatments 2 & 3 had significantly greater penetration resistance than treatments 1 & 4. Generally, the standard organic practice (Trt1) with shallow tillage several times a year plus cultivation for weeds resulted in reduced soil health outcomes and less stored C. Penetration resistance was overall dramatically lower on our reduced and no-tillage organic plots than on an adjacent (20 m away) field with the same soil type that had grown vegetables with standard tillage for 10 years. Research Objective three: contribute to a growing scientific body of knowledge on profitability and ecosystem services. Activities: In early spring 2024, while all plots at CMREC were vegetated with a young rye-hairy vetch cover crop mixture, we conducted simulated rainfalls with distilled water using Cornell sprinkler infiltrometers for 110 mm of runoff. We measured soluble P in every 20 mm increment. We monitored soil water at 2 depths using data loggers. Data: The cumulative P loss (g/ha) was greatest for Trts 1 & 3 (> 200 g/ha) but significantly lower (100-110 g/ha) for Trt2 and 4. The infiltration rate and time to runoff were not affected by treatments. Sediment loss was negligible as all plots had a cover crop growing. Carbon concentration was reduced in Trt1 compared to Trts 3 and 4. Results: Continuous legume-grass hay during the transition period (Trt4) generally showed improved soil health compared to the standard organic with tillage management (Trt1). This relationship was significant for soil respiration, labile and total soil carbon, and aggregation. Publications Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Gairhe, B., and Weil, R. (2023). Transitioning to organic grain production in Maryland: Evaluating soil-health impact of four strategies. International annual meetings of the American Society of Agronomy and the Soil Science Society of America. Indianapolis, IN. 30 Oct. 2023. ASA/CSSA/SSSA. <https://scisoc.confex.com/scisoc/2023am/meetingapp.cgi/Paper/154129> Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: Hirsh, S., S. Dill, B. Beale, E. Crowl, A. Kness, A. Leslie, N. Little, K. Nichols & M. Todd. 2024. Oral presentation -- Cultivating Conservation for Maryland and Delaware's Small and Historically Underserved Farmers. National Association of County Agricultural Agents Annual Meeting and Professional Improvement Conference. Dallas, TX. 16 July 2024. Discussed technical and financial assistance available for conservation practices including transitioning to organic agriculture; programming emphasized resources for Historically Underserved growers. Progress 09/01/22 to 08/31/23 Outputs Target Audience: Scientists researching or interested in organic transitioning or organic farming practices and effects soil health; farmers interested in transitioning or already growing organic crops, along with farm-advisors in private practice or government agencies. Changes/Problems: As originally proposed, during the fourth field season we plan to do most of the soil health and water quality measurements so as to reflect the influence of the previous three-year transition strategies on these properties. Due to the very dry winter season, suction lysimeters did not provide consistent leaching water samples. Therefore, we use several Cornell Sprinkler Infiltrimeters (Van Es and Schindelbeck, 2005) to apply simulated rainfall to each plot during early 2023 and collected both surface runoff water and leachate (using suction lysimeters set at 20 cm under each rain simulation cylinder). These samples are being analyzed for dissolved and total N and P to assess the impact of the transition strategies at four sites on the potential for nutrient losses via surface runoff and leaching. In this fourth year of the study, all plots are treated alike so that the first certified organic crop (food-grade corn) will serve as a bioassay to indicate the effectiveness of the four transition strategy treatments in terms of crop productivity. Once the 2023 corn crop is harvested and data collected, we will still need to process soil, plant, and water samples collected in 2023, analyze the samples, run statistical analyses on the data, and write manuscripts, reports, and extension publications. However, in the Spring of 2023, young corn plants in our field research plots at two sites (LESREC and Farm B) were severely damaged by wildlife (deer at Farm B and raccoons at LESREC). The raccoon damage appeared to be quite uniform throughout the experiment, but the deer damage was greatest on the most vigorously growing plants. Reliable yield data is unlikely to be attainable for these two sites. The Organic and Transition Grain Marketing Directory and the Mentee-Mentor pairing programs were cut short when the main person from Future Harvest working on them, Niamh Shortt, tragically passed away. Future Harvest personnel are working to tie up any loose ends with those programs. On the positive side, we were able to create an alliance between the University of Maryland Dining Services Head Chef, the farm manager for the Central Maryland Research and Education Center, and a Future Harvest farmer and UMD alumnus who has a small feed mill. The farm manager has to agree to harvest, segregate, and deliver an expected 6,000 pounds of food-grade corn from our research plots at CMREC to the farmer-miller-alumnus in Montgomery County who will clean, grind into grits and flour, and bag and deliver the corn products to UMD Dining Services, who, in turn, will serve NIFA-sponsored, Terp-Grown, Terp Processed and Terp-Cooked masa, grits and polenta dishes to UMD Terp students. We hope to get some good extension attention and media coverage for this whole process. What opportunities for training and professional development has the project provided? As part of this project, we are supporting the Ph.D. program for one graduate research assistant, Biwek

Gairhe. We anticipate that he will receive his Ph.D. degree with the conclusion of this project in 2024. We also continue to provide research experience and professional development to nine undergraduate students at the University of Maryland, some of whom are working on aspects of the study for internship or research experience credits and some of whom are being paid hourly. The project is providing them with hands-on first-hand experience with agricultural research, data handling and curation, and soil and plant analysis. This helps them to complete their bachelor of Science degree and giving them a head start on their scientific careers. Finally, we are also educating several farmers through the mentor and extension programs, providing them with professional development. How have the results been disseminated to communities of interest?The project has an active extension component which has conducted online and in-person workshops and informative sessions as well as published several videos on YouTube. Through our relationship with Future Harvest, we have presented our results to a diverse audience of farmers and food activists. We have also kept the Maryland Department of Agriculture, especially the organic certification branch, abreast of our results. What do you plan to do during the next reporting period to accomplish the goals?As stated in the original proposal and the recently approved no-cost extension request, we plan to continue the field research at the four sites where we have replicated experiments through harvest in fall 2023. In the Fall of 2022, we harvested and measured the soybean crop components and planted cover crops to prepare for the 4th cropping season of the project. The first certified organic crop was planted in spring 2023 and is now being grown. During Fall 2023 and Winter 2024 we plan to complete the soil health and water quality measurements so as to reflect the influence of the previous three-year transition strategies on these parameters. In the fourth crop year of the study, all plots are treated alike so that the certified organic corn crop will serve as a bioassay to indicate the effectiveness of the four transition strategy treatments in terms of crop productivity. Late 2023 and early 2024 will be spent processing samples, analyzing data, and writing manuscripts, reports, and extension publications. We plan to graduate the project Ph.D. student in May 2024 and keep the project going through August 2024 to complete the data analysis and produce reports and publications. Impacts What was accomplished under these goals? As of 31 August 2023, our project was in its fourth and final crop season for which the harvest is expected to be certified organic food-grade corn. In fall 2022, we harvested our third transition crop (soybean) grown under three contrasting strategies. Farmers at three sites struggled with a lack of equipment. The farmer at the fourth site has over a decade of experience in organic grain farming and is currently transitioning to organic in a few additional fields, one of which is a project research site. Planting soybeans into tall cover crops using a planter better adjusted for planting into a traditional tilled seed bed resulted in poor stands and little competitiveness against weeds. In plots using no-tillage, we planted soybeans with narrow row spacing (planted in 36 cm wide rows or drilled in 18 cm wide rows). At two sites this narrow row strategy allowed soybeans to close canopy quickly and outcompete weeds. At CMREC all three strategies produced yields ~ 5,500 kg/ha (60-65 bushels/acre), well above the state average. Practical lessons were conveyed to farmers, farm advisors, and government agency personnel in online videos, Zoom panel discussions, and in-person presentations. Farmers will be able to avoid making costly mistakes highlighted by our research. Our results at some sites give hope to farmers interested in maintaining soil health with minimal soil disturbance. Research objective 1. Provide farmers with practical information about agronomic and economic performance and management challenges\... Activities. We completed the third year (soybean) and began the fourth and final project year (certified organic corn) for replicated field experiments at four sites. Data. Fall 2022 soybean yields and seed weights were determined. Cover crop ground cover and biomass were determined in Fall 2022 and Spring 2023 just before termination. In Spring 2023 corn crop stand establishment and weed biomass were measured in July. Results. Actual stands ranged from 450,000 to as low as 50,000 plants per hectare, with the best stands in trt1 with full tillage at one site (experienced organic farmer), but no effect of treatment on soybean stands or yields at another site with the highest overall yields. At the experienced organic farmer site, stands within a treatment were highly variable due to wet soils, Canada geese damage and especially planter settings inappropriate for heavy cover crop biomass. Where the crop stand and soybean stands were poor, weed pressure became quite high. Final soybean yields varied from 1,000 to 6,800 kg/ha. At one site yields were higher in trt 1, but in another where soybeans were drilled in narrow rows all three treatments yielded about 6,500 kg/ha. Soybean stand density in July accounted for 82% of the variability in combine-harvested soybean yields. All four strategies could produce high yields under some conditions, but that achieving uniform crop stands was a critical challenge. By the end of August 2023 the organic corn was filling grain and yield potential looked good at two of our sites, but the other two sites had poor stand establishment and damage by deer and raccoons. Key outcomes. Farmers learned how transitional soybean yields were closely related to careful planter adjustment and the use of narrow rows to establish high-density weed-competitive stands in cover-cropped soils. Research objective 2): how transition systems affect soil health. Activities. Soil health impact sampling was begun in August 2022 and soil health measurements will continue to be made in the field or on samples obtained in 2023 which will reflect the three previous field seasons during which the four experimental strategies were implemented. Data collected. In the fall of 2022, we measured aggregate slaking stability and the 24-hour and 72-hour CO₂ burst activity (soil respiration) as a biological measures of soil health. We buried the tea bags in June 2022 at three sites and they were retrieved after 90 days in Sept. 2022. We measured fractional

dry matter loss for each type of tea, as well as the calculated stability factor (a) and decay rate (k) according to Keuskamp et al. 2013. Soil samples taken from 0-15 and 15-30 cm were collected in June 2023 for pre-side dress nitrate test (PSNT) to aid in assessing N fertility needs. The 0-15 cm samples were also sent for routine soil tests (Mehlich3 nutrient extraction and loss on ignition soil organic matter). Results. Across 3 sites and 3 treatments, the tea bag stability factor (a) was significantly lower in the full tillage treatment No. 1 compared to reduced tillage treatments No. 2 and 3. Averaged across the three sites for which samples were available, Trt #1 with traditional tillage had lower rates of microbial activity (both 24-hour and 72-hour soil respiration) than the less-disturbed treatments. Trt #1 also had significantly lower aggregate stability scores averaged across three sites, and in particular in silty soils at LESREC. Soil active carbon (POXC) was not affected by treatment at most sites but was significantly lower in Trt #1 than the less disturbed TRTS 23 at Farm A. The June 2023 PSNT indicated that despite the heavy hairy vetch-rye cover crop, most treatments had too little soil nitrate for optimal corn yields. The 0-15 cm samples from June 2023 revealed that after 2.5 years, the low-disturbance Treatments #2 & #3 had ~7% higher soil organic matter than the traditional tillage Treatment #1 (2.5% v 2.3%), averaged across all four sites. 4) Key outcomes. Farmers and researchers learned about indicators of soil health and how levels of soil disturbance and cover cropping intensity affect soil health. Research objective 3: Profitability and ecosystem services. 1) Major activities completed / experiments conducted. The lack of activity in economic analysis is partially because our collaborating economist, Dr. Dale Johnson, was on sabbatical leave in Kenya. 2) Data collected. All input and operational costs are being recorded for all sites, and either actualized returns or standard market value returns will be used to calculate partial Enterprise budgets. 3) Summary statistics and discussion of results. No economic results to report. 4) Key outcomes or other accomplishments realized. No economic outcomes for this reporting period Extension and outreach objectives. Activities. The program that paired experienced organic grain farmers with farmers wishing to transition but without organic experience set up two mentor-mentee relationships. Expansion of this program as well as field days was tragically limited by the sudden death of our main collaborator from Future Harvest, Niamh Shortt. The project PI and lead Ph.D. student presented, at the annual Future Harvest conference in January 2023, an extension workshop focused on managing the transition to organic grain farming. Data collected. We are not collecting data on human subjects. Results. The project paired farmers wanting to transition to organic grain with two recognized, experienced organic grain producers, and online extension videos and workshops received over 200 views. Key outcomes. Underserved and inexperienced farmers wanting to transition to organic grain production underwent changes in knowledge when the participants in the workshops and mentoring program learned about alternative management approaches for the transition. They were able to consider managing soil with less disturbance and using crop competition and cover crops for weed suppression Research Summary here: Transition to Organic Grain-Summary of Agronomic and Soil Health Results 9/2022 - 8/2023: <https://drive.google.com/file/d/1ySCVC4JWDIVlyH4XGB9MOYN13A6XYsC/view?usp=sharing> Publications Type: Other Status: Published Year Published: 2023 Citation: Weil, R., and Gairhe, B. 2023. Planting green then rolling vetch-rye w cultipacker for organic corn Organic Transition Project Funded by USDA/NIFA, University of Maryland, <https://www.youtube.com/watch?v=BLutWkqjmOI> Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Gairhe, B., and Weil, R. (2022). Strategies to transition to organic grain: Impacts on soil health. Communication and public engagement for healthy people and a healthy planet, the International Annual Meeting of the American Society of Agronomy and the Soil Science Society of America. Baltimore, Maryland. 6-9 NOVEMBER 2022 ASA and SSSA. <https://scisoc.confex.com/scisoc/2022am/meetingapp.cgi/Paper/145841> Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Weil, R., and Gairhe, B. (2023). Final year of organic grain transition: Impacts on soil health and crop productivity. A Time to Grow: Regenerative Farming for People & the Planet. College Park, Md. 13 January 2023 Future Harvest - Chesapeake Alliance for Sustainable Agriculture. <https://futureharvest.org/wp-content/uploads/2023/01/January-2023-Program-Final-No-Marks.pdf> Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Weil, R., and Gairhe, B. (2023). Transitioning to organic grain production: Strategies to maximize profitability and ecosystem services while reducing risks and barriers. USDA/NIFA Organic Project Directors Meeting. Washington, DC. Poster. https://drive.google.com/file/d/1E71YRI3wl_dpdxpu4_IV6BLmtpzaB1E-/view?usp=sharing Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Weil, Ray. 2022. Measuring Soil Health- Which Tests Provide the Most Useful Information? Delmarva Soil Summit. Feb. 7, 2022, Salisbury, MD. In-person and Virtual audience of ~200. <https://viewstub.com/watch/2022-Delmarva-Soil-Summit>. Use access code: 74C5DFCE Type: Other Status: Published Year Published: 2023 Citation: Weil, R., and Gairhe, B. 2023. Organic regenerative farming #2: Soil mulched & full of life as organic no-till corn emerges. <https://www.youtube.com/watch?v=qkbJikFpaF4> Type: Other Status: Published Year Published: 2023 Citation: Weil, R., and Gairhe, B. 2023. Regenerative organic corn no-till planting green without chemicals. Organic Transition Research Funded by USDA/NIFA, <https://www.youtube.com/watch?v=nc9WbjVyzZo> Progress 09/01/21 to 08/31/22 Outputs Target Audience: Scientists researching or interested in organic transitioning or organic farming practices and effects soil health; farmers interested in transitioning or already growing organic

crops, along with farm-advisors in private practice or government agencies. Changes/Problems:As originally proposed, during the fourth field season we plan to do most of the soil health and water quality measurements so as to reflect the influence of the previous three-year transition strategies on these properties. Due to the very dry winter season, suction lysimeters are not providing consistent leaching water samples. Therefore, we plan to use several Cornell Sprinkler Infiltrimeters (Van Es and Schindelbeck, 2005) to apply simulated rainfall to each plot during early 2023 and collect both surface runoff water and leachate (using suction lysimeters set at 20 cm). These samples will allow us to assess the impact of the transition strategies at four sites on the potential for nutrient losses via surface runoff and leaching. In this fourth year of the study, all plots will be treated alike so that the certified organic corn crop in 2023 will serve as a bioassay to indicate the effectiveness of the four transition strategy treatments in terms of crop productivity. Once the 2023 corn crop is harvested and data collected, we will still need to process soil, plant, and water samples collected in 2023, analyze the samples, run statistical analyses on the data, and write manuscripts, reports, and extension publications. Carrying out this critical climax of the project will require an additional 1-year no-cost extension (beyond the current August 31 2023 end date) to fund the Ph.D. student through graduation in May 2024 and keep the project going long enough to complete the data and produce the reports and publications. What opportunities for training and professional development has the project provided?As part of this project, we are supporting the PhD program for one graduate research assistant, Biwek Gairhe. We anticipate that he will receive his PhD degree with the conclusion of this project in May 2024. We also are providing research experience and professional development to nine undergraduate students at the University of Maryland, some of whom are working on aspects of the study for internship or research experience credits and some of whom are being paid hourly. The project is providing them hands-on first-hand experience with agricultural research, data handling and curation, and soil and plant analysis. This is helping them to complete their bachelor of Science degree and giving them a head start on their scientific careers. Finally, we are also educating several Farmers through the mentor and extension programs providing them professional development. How have the results been disseminated to communities of interest? The project has an active extension component which has conducted online and in-person workshops and informative sessions as well as published several videos on YouTube. Through our relationship with Future Harvest we have presented our results to a diverse audience of farmers and food activists. We have also kept the Maryland Department of agriculture, especially the organic certification branch, abreast of our results. As an example, here's the Extension announcement for the December 10, 2021, virtual webinar: <<https://drive.google.com/file/d/1KSX8wf4GVKQT4K72D0YWZhGbVppVuVWm/view?usp=sharing>>. What do you plan to do during the next reporting period to accomplish the goals? As stated in the original approved proposal, we plan to continue through harvest in fall 2023 the field research at the four sites where we have replicated experiments. In the Fall of 2022, we harvested and measured the soybean crop components and planted cover crops to prepare for the 4th cropping season of the project. The first certified organic crop will be grown in spring 2023. During 2023 we plan to do most of the soil health and water quality measurements so as to reflect the influence on these properties of the previous three-year transition strategies. In this fourth year of the study, all plots will be treated alike so that the certified organic corn crop in 2023 will serve as a bioassay to indicate the effectiveness of the four transition strategy treatments in terms of crop productivity. Late 2023 and early 2024 will be spent processing samples, analyzing data, and writing manuscripts, reports, and extension publications. Carrying out this critical climax of the project will require an additional 1-year no-cost extension to fund the Ph.D. student through graduation in May 2024 and keep project going long enough to complete the data and produce the reports and publications. Impacts What was accomplished under these goals? Impact of our project. We aim to compare alternative strategies for managing crops and soils during the organic transition period. As of August 2022, our project was in its third crop season. During this reporting period, we harvested our second organic crop (corn) grown under three contrasting strategies from typical full tillage to innovative no-tillage organic with intensive cover cropping. The collaborating farmers and research station managers encountered many problems that conventional grain farmers thinking of converting to organic are likely to face. Most conventional no-till grain farmers have little cultivating, tillage, roller crimping, or hay-making equipment. Transitioning to organic is likely to be tried on just a field or two at first, making it difficult to justify investing in expensive equipment. Farmers at three sites struggled with lack of equipment while the fourth is an experienced organic farmer who is transitioning a few additional fields. Planting into a heavy cover crop using a planter adjusted to plant into a traditional tilled seed bed resulted in poor stands in the low disturbance treatments. Poor crop stands resulted in little competitiveness against the weeds. Such practical lessons were conveyed to farmers, farm advisors, and government agency personnel in online videos, Zoom panel discussions, and in-person presentations. Armed with this information, farmers will be able to avoid making costly mistakes highlighted by our research. Our results also gave hope to farmers interested in maintaining minimal soil disturbance as at some sites, low-disturbance low-cost systems produced very respectable yields. Research objective 1. Provide farmers with practical information about agronomic and economic performance and management challenges\... 1) Activities. We completed the second year (corn) and began the third year (soybean) for replicated field experiments at four sites. 2) Data. Fall 2021 corn yields and seed weights were

determined. Cover crop ground cover and biomass were determined in Fall 2021 and Spring 2022 just before termination. Spring 2022 soybean crop stand establishment and weed biomass were measured in July. 3) Results. Actual stands ranged from 80,000 to as low as 20,000 plants per hectare, with the best stand in trt1 with a full tillage. Stands were highly variable due to wet soils, Canada geese damage and especially planter settings inappropriate for heavy cover crop biomass. Where the crop stand was poor, weed pressure became quite high. Final corn yields varied from 12,000 to 1,000 kg/ha. At three sites yields were higher in trt 1, but in one site trt 3 had the highest corn yields. A regression tree showed that the main factor affecting yield was plant stand density. Secondly, weed dry matter exceeding 4,350 kg/ha predicted very low yields. We concluded that all four strategies could produce high yields under some conditions, but that achieving uniform crop stands was a critical challenge. 4) Key outcomes. Farmers learned how corn yields were closely related to careful planter adjustment. Research objective 2): how transition systems affect soil health. 1) Activities. Soil health impact sampling was begun in August 2022 but most soil health measurements will be made in the field or on samples obtained in 2023 which will reflect the three previous field seasons during which the four experimental strategies were implemented. 2) Data collected. In 2022 the baseline samples collected during 2020 were analyzed for bulk density and permanganate oxidizable carbon (POXC) as well as soil chemical tests. In addition, during summer of 2022 we buried 88 pairs of red and green tea bags (Keuskamp et al., 2013) at 8 cm depth as indicators of microbial decomposition dynamics. Red tea (Rooibos leaves) is a difficult-to-decompose plant tissue with many aromatic compounds. Green tea is much easier to decompose. When the two types of tea bags are buried in pairs, the difference in decomposition rates can be used to derive the decomposition rate and litter stabilization factor. We buried the tea bags in June 2022 and they were retrieved in Sept. 2022. 3) Results. Soil bulk density increased with depth at all sites, and was lower in treatment 1 at one site, but this was due to recent tillage. The POXC values were markedly different among sites and lower with depth. At the Lower Eastern Shore commercial organic farm trt 1 (full tillage), had lower levels of POXC at 0-10, 10-20 and 20-30 cm depths. 4) Key outcomes. Farmers and researchers learned about indicators of soil health. Economic viability objectives. 1) Major activities completed / experiments conducted. During the reporting time frame for this progress report we did not perform economic analyses. The lack of activity in economic analysis is partially because our collaborating economist, Dr. Dale Johnson, was on sabbatical leave in Kenya. 2) Data collected. All input and operational costs are being recorded in all sites, and either actualized returns or standard market value returns will be used to calculate partial Enterprise budgets. 3) Summary statistics and discussion of results. No economic results to report. 4) Key outcomes or other accomplishments realized. No economic outcomes for this reporting period. Research objective 3: \...profitability and ecosystem services \... 1) Activities. These activities will occur during the final cropping season of the project. 2) Data. NA 3) Results. NA 4) Key outcomes. NA Extension and outreach objectives. 1) Activities. The mentor - mentee program that paired experienced organic grain farmers with farmers wishing to transition but without organic experience set up two of these relationships. Expansion of this mentor - mentee program as well as Field Days was tragically limited by the sudden death of our main collaborator from Future Harvest, Niahm Shortt. We conducted several virtual extension events including a farmer panel discussion under the auspices of Future Harvest's annual conference. The PI made an extension visit on 08 July 2022 to Purple Mountain Organic grain farm (an African American small grain farm) and University of the District of Columbia Farm (an Historically Black College) to assist with organic corn and rice grain production practices. 2) Data collected. We are not collecting data on human subjects. 3) Results. The project paired farmers wanting to transition to organic grain with two recognized, experienced organic grain producers and online extension video and workshops received over 200 views. 4) Key outcomes. Underserved and inexperienced farmers wanting to transition to organic grain production underwent changes in knowledge when the participants in the workshops and mentoring program learned about alternative management approaches for the transition. They were able to consider managing soil with less disturbance and using crop competition and cover crops for weed suppression. Summaries of the results can be found in these URLs: Results for Soybean and soil health, Jan to Aug 2022 - https://drive.google.com/file/d/19XcfFd2HnYkl5yjIM_04509L91QZm1nR/view?usp=sharing Results for Corn Sept. to Dec. 2021 - https://drive.google.com/file/d/1JWU8Hnv_3ha9fX2-289loXDSiWVKH4hW/view?usp=sharing Publications Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Weil, R.R., B. Gairhe and S. Hirsh. (2021) Transitioning to organic grain production: Strategies to maximize profitability and ecosystem services while reducing risks and barriers. ASA, CSSA, SSSA International Annual Meeting - A Creative Economy for Sustainable Development, Salt Lake City, Utah. 09-11 Nov. 2021. American Society of Agronomy and Soil Science Society of America. <https://scisoc.confex.com/scisoc/2021am/meetingapp.cgi/Paper/136882> Type: Other Status: Published Year Published: 2022 Citation: Weil, Ray. 2022. Measuring Soil Health- Which Tests Provide the Most Useful Information? Delmarva Soil Summit. Feb. 7, Salisbury, MD. In-person and Virtual audience of ~200. <<https://viewstub.com/watch/2022-Delmarva-Soil-Summit>>. Use access code: 74C5DFCE Type: Websites Status: Published Year Published: 2021 Citation: Weil, R., Gairhe, B., and Hirsh, S. (2021). Interested in \$10 corn and \$30 soybeans for certified organic, but not sure how to transition? Agronomy News 12:21-24. <https://extension.umd.edu/resource/interested-10-corn-and-30-soybeans-certified-organic-not-sure-how-transition>

Type: Other Status: Published Year Published: 2021 Citation: Weil, R., Hirsh, S., and Shortt, N. 2021. New lessons learned in organic grain transitions. University of Maryland Extension, Agriculture and Food Systems, <https://www.youtube.com/watch?v=USVur-GAI0o&t=5910s> **Progress** 09/01/20 to 08/31/21 **Outputs** Target Audience: The primary audience consists of innovative grain (corn, soybean, wheat, barley, etc.) farmers interested in transitioning all or parts of their farms to organic certified production, and scientists who study organic farming systems. The immediate focus is on such farmers and scientists working in the mid-Atlantic region, but research will be largely applicable to farms in much of the East Coast, upper South, and mid-West, as well. Secondary target audience members include farm advisors and farm managers on commercial and research farms who desire to gain knowledge of organic and transitional practices and impacts. Additionally, our target audience will include members of the public and government who are interested in the issues of organic food and soil health. Changes/Problems: One of the project key personnel, Nathaniel Richards, left Maryland Extension and the project in 2021 and we will try to replace him with another extension agent in a grain-producing county. However, our biggest problem for the project - and also for transitioning farmers - is probably the lack of specialized equipment for farm operations unique to organic farming, especially equipment used for mechanical weed control such as high-residue, low-disturbance cultivators. Because most non-organic Maryland grain farmers use no-till techniques, both research stations and commercial grain farmers in our state typically lack specialized equipment for tillage and cultivation. While experienced organic grain farmers acquire a complement of machinery, farmers considering transitioning to organic one or two fields at a time find it difficult to economically justify the purchase of such equipment. In spring 2020, we conducted a preliminary trial of a new organically approved herbicide that is based on eugenol, an essential oil of the clove plant. We conducted this trial to determine whether this OMRI-approved herbicide would be useful in terminating cover crops to facilitate no-till planting in the organic transition system that was designed to minimize disturbance and maximize cover cropping and soil health. The results of this preliminary trial showed that the herbicide (brand named Weed Slayer®) was highly effective against grassy weeds with effects similar to glyphosate, but only partially effective against broadleaf species, such as vetch and clover, used for cover cropping. Unfortunately, in December 2020 the California Department of Food and Agriculture came out with a warning that they had found both glyphosate and paraquat in the AGRI-Gold sticker part of the two parts that are mixed to make Weed Slayer. Although we inquired extensively, we were never able to find out the concentrations that they found and get the lab data because they said there was a lawsuit involved. So, we don't know if these contaminants were there in high enough amounts to have herbicidal activity or whether they were in amounts that might have just been accidental contamination. It was a pretty big set-back to not be able to use the Weed Slayer for weed control and cover crop termination as planned for some of the research treatments. We did buy another product called Suppress that was much less effective. Nonetheless, the corn in several of the treatments is doing quite well at most sites, despite a fair amount of weed pressure. No Cost Extension The project was originally written and approved with 4 years of activity but only 3 years of funding. This was necessary as, by definition, the organic transition period is a full 3 years. The research was set up to use the crop growth and yields in the first year of certified organic grain production (by definition, the 1st year after the transition period and the 4th year of the study), as a critical biological indicator of the success of the four different 3-year transition strategies. Therefore, there was a need for a no-cost extension of one year built into the project design and timeline. However, it is now apparent that we will need to request a 1.7-year extension to the project for the reasons outlined here. First, although we were initially notified of the decision to fund our proposal in late July 2019, we were told not to make the grant public until mid-October 2019. Funds also did not become available for us to use until late October 2019. This timing meant that the initiation of the grant activities was hampered in several ways causing us to lose most of the full first year of cropping activities. We were not able to begin recruiting farmers until late October and held our first meeting with farmers in January. We have now established two pairs of mentee-mentor relationships and do have two collaborating farmers who are managing replicated research plots on their farms. The research proposal called for treatments to be applied in the field during August 2019 to establish cover crops and forage seedings to prepare for the coming spring 2020 as the 1st crop year of the 3-year transition period. Because of the timing factors mentioned above, it was not possible to begin the project treatments as planned in August-September 2019. However, we were able to adjust our plans and plant spring oats in April 2020, thus preventing us from losing the entire 2020 cash crop season. The cover crop and perennial forage planting treatments originally planned to begin in August of 2019 were established in August of 2020. This obviously put us behind by one year as compared to the original plan that assumed funding would begin July 1st and fieldwork could begin in August of 2019. The second reason for the need for a 1.7-year extension is the impact of the Covid-19 pandemic on our project activities. For most of the year from mid-March 2020, Maryland was put on lockdown and the University of Maryland campus and laboratories were closed. This meant that our normal research workforce of undergraduate students is no longer available to us. While vaccines brought reduced Covid-19 risks in the first quarter of 2021, cases and risks, and restrictions increased again in the second quarter of 2021. We are moving ahead with research as best we can in the field, the Covid-19 limitations are still restricting what we are able to do. Therefore, in order to meet the project objectives, we anticipate that we will need an extra

9 months on top of the initially planned 1-year extension. What opportunities for training and professional development has the project provided? One Ph.D. student is supported in the Graduate School as a GA by the project and is gaining comprehensive experience and training in research design and implementation. He has also had the opportunity to write a research grant proposal (graduate student grant from NE SAFE), write and submit an abstract for a paper to be presented to the Agronomy Society of America national meetings, and prepare presentations for an extension field day planned for September 2021. Seven undergraduate students have assisted in conducting the research and have gained experience in everything from field experiment layout, to soil sampling, to lab analysis and data management. How have the results been disseminated to communities of interest? The early results have been disseminated via a conference panel discussion at the annual Future Harvest conference which was attended by 700 + farmers and food system professionals. In addition, an abstract for a scientific presentation has been submitted to the Agronomy Society of America for their November 2021 international meetings. What do you plan to do during the next reporting period to accomplish the goals? Activities Planned to Meet Research Objectives: Analyze baseline soil samples from all treatments and sites for remaining physical, chemical, and biological properties. Measure and analyze corn yields from trt 1, 2, and 3 Measure hay yields and forage quality. Collect soil samples after harvest of corn to investigate the difference in soil properties among different treatments. Continue measuring the soil temperature, moisture, EC and other soil parameters in the field to elucidate treatment effects and mechanisms. Establish cover crop and tillage treatments after harvest of corn, analyze cover crop plant samples to assess their contribution to soil fertility. Plant Soybean in trt 1, 2 and 3 in spring 2021 using adaptive research practices Continue collecting operational and input expenses and income for economic analysis. Activities Planned to Meet Extension objectives: Publish at least one extension article to disseminate information about the organic transitions study and early findings/lessons. Organize at least one farmer's field day in one of our experiment sites. The first field day has been planned for September 2021. Publish a beta version of the Grain Marketing Guide. Continue mentor-mentee activities for the two established relationships. ****Impacts**** What was accomplished under these goals? Field Research experiments have been established at four locations, two on experiment stations and two on commercial farms. The four treatments are conceptually defined and are shaped by adaptive research decisions made in collaboration with the two commercial farmers and two research farm managers. The experiment consists of three or four treatments (depending on the site) along a continuum of tillage intensity, cover crop optimization, and purchased inputs to be compared during the three-year transition period (starting with spring of 2020) and concluding with a certified organic corn crop in summer 2023. The treatments are Traditional full-tillage, full input, organic oat-corn-soybean-corn, with simple cover crops and input substitution. Reduced-till, moderate input, oat-corn-soybean-corn with high-biomass, early-planted cover crops terminated by roller-crimping. Minimum-till, minimum input, oat-corn-soybean-corn rotation with early-planted, precision-zoned high biomass diverse cover crops terminated by roller-crimping. Perennial alfalfa-grass hay, untilled, implemented in experiment stations only. Limed and fertilized with, manure and rock powders. Due to the lack of hay-making equipment owned by the commercial farmers or available locally to borrow or rent, the fourth treatment was established only on the two experiment stations. Field Research Accomplishments (August 2020-July 2021) August, 2020 - Planted summer cover crops in Trt 3 at CMREC, LESREC, and Cooper sites - Baseline soil sampling at CMREC, Cooper and LESREC sites - Calculation of bulk density of baseline soil samples - Subsampling and storage of baseline soil samples at -20 degrees C for later DNA analysis, rest of the samples were air dried and processed for further analysis September, 2020 - Biomass sampling of summer cover crops in Trt 3 from three locations per plot before termination, the samples were dried, taken dry weight, ground and stored. - Termination of summer cover crops in CMREC, LESREC, and Cooper sites. - Planting of cover crops in Trt 1, 2 and 3 at CMREC, Cooper and LESREC sites. - Planting of alfalfa-grass in Trt 4 at CMREC and LESREC. October, 2020 - Baseline soil sampling at Cavanaugh's site. - Planting of cover crops in Trt 1, 2 and 3 at Cavanaugh's site. November, 2020 - CANOPEO analysis of cover crop establishment at CMREC, LESREC, and Cavanaugh's site December, 2020 - Biomass collection of cover crop samples, the samples were dried, taken dry weight, ground and stored. January, 2021 - Collection of operations, inputs, expenses details from all four sites - Meeting with the organic transitions team for a discussion of accomplishments, issues, challenges, and future plans - Installation of soil temperature, moisture, and EC dataloggers at Cooper (2 loggers and 12 sensors) and LESREC (3 loggers and 18 sensors) - Attended Future Harvest's 22nd annual conference at which three project participants and the PI presented preliminary results and lessons learned at a panel discussion. February, 2021 - Installation of soil temperature, moisture, and EC dataloggers CMREC (4 loggers and 24 sensors) March, 2021 - Attended soil health innovations conference. Adaptive management discussions among researchers and four farm managers took place to develop specific practices for the four transition strategies adapted to the conditions at each site. April, 2021 - Installation of soil temperature, moisture, and EC dataloggers at Cavanaugh's (2 loggers and 12 sensors) - Submission of a research grant to NE-SARE titled "How the transition to Organic Grain Effects Biological Indicators of Soil Health" to leverage additional funds to allow exploration of the transition treatments' effects on soil metagenomics. May, 2021 - Collection of cover crop biomass samples from all four sites. The samples were dried, taken dry weight, ground, and stored. - Organic Termination of cover

crops in Trt 1, 2 and 3 at CMREC, Cooper and LESREC by tillage, mowing, and/or roller-crimping. Precision bio-strip till cover crop was highly successful LESREC. - Harvesting of hay from trt 4 at LESREC - Planting of Blue River organic corn hybrid in Trt 1, 2, and 3 (no-till) at CMREC, Cooper and LESREC sites June, 2021 - Planting of Blue River corn hybrid in Trt 1, 2 and 3 (no-til) at Cavanaugh site - Assessment of corn establishment at all four sites - LECO analysis of cover crop samples for measuring percentage of C and N - Soil sampling for Pre-Sidedress Nitrate test (PSNT) analysis at V5 stage of corn at CMREC, LESREC and Cooper sites, accompanied by rapid nitrate extraction and analysis using a Horiba potentiometric ion-selective Nitrate Meter. July, 2021 - Soil sampling for Pre-sidedress Nitrate test (PSNT) analysis at V5 stage of corn at Cavanaugh site, PSNT analysis using Horiba Nitrate Meter - Assessment of weeds in all treatments - Harvesting of Hay from Trt 4 at CMREC Future Harvest and Extension Accomplishments. Future Harvest committed to matching three farmers new to the transition process with three farmer consultants well versed in the process of converting from conventional to organic grain production. Additionally, Future Harvest committed to running 2 workshops (one on Delmarva and one on the western side of the Chesapeake Bay) on organic grain transition. Thus far, Future Harvest has made two of the three mentor-mentee matches proposed. It turned out that while a number of farmers were interested in growing organic grain, few were willing to make the switch from conventional to organic due to lingering questions about the finances of the process. This is a valid and realistic concern given the narrow profit margins farmers face, the different equipment needs from conventional to organic (hence possibly high transition costs as farmers may have to purchase new equipment), the uncertainty of their ability to find buyers willing to pay a premium for their transitional grains during the 3 yr transition process, and general questions about the market conditions for organic grain locally. Future Harvest partnered with Extension to run a workshop called "Ask A Farmer -- Organic Grain, Does It Pay?". This workshop consisted of a panel discussion and Q&A space to allow farmers to identify whether a transition is a good fit for them and if so to get connected into our Future Harvest's Great Grains Project. Farmers Aaron Cooper of Cutfresh Organics and Steve Krazowski of Mason's Heritage Farm were the farmer panelists answering conventional growers' questions about organics. Extension agent Shannon Dill was on the panel to answer financial questions and extension agent Sarah Hirsh both facilitated the session and answered questions about soil health. The second Future Harvest workshop is being organized in partnership with the Common Grain Alliance. As the Organic Transitions project research moved forward in 2020, the research team decided to integrate a small grain into the crop rotation (oats). As we searched for premium buyers of these oats, we've learned much along the way. We learned that the brewer community is interested in purchasing local oats, but that they have very specific types and quantities they need. For this second Future Harvest workshop, we will bring a local brewer together with a local farmer to model the conversation about requirements and preferences in the buying and selling process so that members of the research, farming, and brewing communities know how to meet each other's needs. Lastly, Future Harvest has been working on creating a directory of buyers for local, transitional grain. This process is well underway and continues with the assistance of three University of Maryland undergraduate students working for the project.

****Publications****

PROGRESS

2019/09 TO 2020/08 Target Audience: The primary audience consists of innovative grain (corn, soybean, wheat, barley, etc.) farmers interested in transitioning all or parts of their farms to organic certified production. The immediate focus on such farmers in the mid-Atlantic region, but research will be largely applicable to farmers in much of the East Coast, upper South, and mid-West, as well. Secondary target audience members include farm advisors and farm managers on commercial and research farms who desire to gain knowledge of organic and transitional practices and impacts. Additionally, our target audience will include members of the public and government who are interested in the issues of organic food and soil health. Changes/Problems: The project was originally approved with 4 years of activity but only 3 years of funding because the program was funding 3-year projects but, by definition, the organic transition period itself is a full 3 years. The research was set up to use crop growth in the first year of certified organic grain production (by definition, the 4th year of the study), as a critical biological indicator of the success of the 3-year transition strategies. Therefore, a no-cost extension of one year was built into the project design and timeline. However, it now appears that an additional 9 months will be required to complete the project due to circumstances beyond our control during the initial phases. First, although we were notified of the decision to fund our proposal in late July 2019, we were told not to make the grant public until mid-October 2019, and funds were not available to us until late October 2019. This timing meant we were not able to begin recruiting farmers until late October. The research proposal called for treatments to be applied in the field during August 2019 to establish cover crops and forage seedings to prepare for the coming spring 2020 as the 1st crop year of the 3-year transition period. Because of these timing factors we were not able to begin the project treatments as planned in August-September 2019. However, we adjusted our plans and planted spring oats in April 2020, thus preventing us from losing the entire 2020 cash crop season. The cover crop and perennial planting treatments originally planned for August-October of 2019 will be established in August-October

of 2020. This put us behind by 1 year as compared to the original plan that assumed funding would begin July 1st and fieldwork could begin in August of 2019. Secondly, as of mid-March, the COVID pandemic closed the University of Maryland campus, and laboratories until mid-summer. While we were able to obtain "essential worker" designation for a few people, our normal research workforce of undergraduate students was (and is) largely not available. The COVID-19 limitations severely restrict what we can do, presumably until sometime in 2021 when vaccines may alleviate the pandemic. The University is gradually opening campus labs, but as of mid-August, we have limited occupancy and no undergraduate students allowed in campus labs. What opportunities for training and professional development has the project provided? The project recruited and hired a graduate research assistant Ph.D. student, Biwek Gairhe, whose assistantship began 02 August 2020. He will pursue a Ph.D. degree with his dissertation centered on this project. He began doing actual work on the project on 15 July 2020. In addition, four undergraduate students (Psurek, Godbey, Owens and Layer) have been involved in field and online work for the project. Covid-19 restrictions have prevented them from working in the on-campus lab setting, but they have been gaining valuable research experience under the mentorship of the PI (Weil) and the Research Assistant (Gairhe). How have the results been disseminated to communities of interest? A report was published in Agronomy News regarding the organic herbicide study. What do you plan to do during the next reporting period to accomplish the goals? Research Objectives: All four treatment planting will be established in the field by October 2020. The perennial hay will be seeded for treatment #4 in late August. Treatments 1, 2, and 3 will receive cover crops of varying intensity to prepare for a corn planting in spring 2021. Treatment #1 will be tilled and mowed to control weeds subsequent to oat harvest and a monoculture legume cover crop (winter peas) planted in late September. Treatment #2 will receive some tillage and an early planted mixed species cover crop sown in early September. Treatment #3 already has a multi-species cover crop sown in July which will be terminated by close mowing and a second early fall cover crop planted without soil disturbance, if possible. This cover crop will consist of rows of radish planted 72 cm (30") apart where the corn rows will be in spring, and a mixture of crimson clover and rye drilled in the middles. Baseline soil samples were collected from at 0-10, 10-20 and 20-30 cm depth in all treatment plots at all 4 sites in July and August. These samples will be processed and analyzed for soil health parameters during the fall and winter of 2020 -2021. The biomass and C/N ratio of the cover crops will be determined in late fall and at spring termination. Weed, pest and fertility issues will be assessed. The economic data collected through the end of the cover cropping season will be analyzed. Extension objectives: Therefore FH plans to partner with UMD Extension on an "Ask A Farmer -- Organic Grain, Does It Pay?" workshop with a panel to allow farmers to identify whether a transition is a good fit for them and if so to get connected into our Project. Farmers Aaron Cooper of Cutfresh Organics and Steve Krazowski of Mason's Heritage Farm will be our farmer panelists answering conventional growers' questions about organics. Extension agent Shannon Dill will be on the panel to answer financial questions and extension agent Sarah Hirsh will both facilitate the session and answer questions about soil health. The second Future Harvest workshop is being organized in partnership with the Common Grain Alliance. As the Great Grains Project research moved forward, the research team decided to integrate a small grain into the crop rotation (oats). As we've searched for buyers of these oats, we've learned much along the way. We discovered the brewer community is interested in purchasing local oats, but that they have very specific types and quantities they need. For this second Future Harvest workshop, we will bring a local brewer together with a local farmer to model the conversation of what each of their needs are in the buying and selling process so that members of the research, farming, and brewing communities know how to meet each other's needs. Lastly, Future Harvest has been working on creating a directory of buyers for local, transitional grain. This process is well underway and continues.

2020/09 TO 2021/08 Target Audience: The primary audience consists of innovative grain (corn, soybean, wheat, barley, etc.) farmers interested in transitioning all or parts of their farms to organic certified production, and scientists who study organic farming systems. The immediate focus is on such farmers and scientists working in the mid-Atlantic region, but research will be largely applicable to farms in much of the East Coast, upper South, and mid-West, as well. Secondary target audience members include farm advisors and farm managers on commercial and research farms who desire to gain knowledge of organic and transitional practices and impacts. Additionally, our target audience will include members of the public and government who are interested in the issues of organic food and soil health. Changes/Problems: One of the project key personnel, Nathaniel Richards, left Maryland Extension and the project in 2021 and we will try to replace him with another extension agent in a grain-producing county. However, our biggest problem for the project - and also for transitioning farmers - is probably the lack of specialized equipment for farm operations unique to organic farming, especially equipment used for mechanical weed control such as high-residue, low-disturbance cultivators. Because most non-organic Maryland grain farmers use no-till techniques, both research stations and commercial grain farmers in our state typically lack specialized equipment for tillage and cultivation. While experienced organic grain farmers acquire a complement of machinery, farmers considering transitioning to organic one or two fields at a time find it difficult to economically justify the purchase of such equipment. In spring 2020, we conducted a preliminary trial of a new organically approved herbicide that is based on eugenol, an essential oil of the clove

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So, we don't know if these contaminants were there in high enough amounts to have herbicidal activity or whether they were in amounts that might have just been accidental contamination. It was a pretty big set-back to not be able to use the WeedSlayer for weed control and cover crop termination as planned for some of the research treatments. We did buy another product called Suppress that was much less effective. Nonetheless, the corn in several of the treatments is doing quite well at most sites, despite a fair amount of weed pressure. No Cost Extension The project was originally written and approved with 4 years of activity but only 3 years of funding. This was necessary as, by definition, the organic transition period is a full 3 years. 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This obviously put us behind by one year as compared to the original plan that assumed funding would begin July 1st and fieldwork could begin in August of 2019. The second reason for the need for a 1.7-year extension is the impact of the Covid-19 pandemic on our project activities. For most of the year from mid-March 2020, Maryland was put on lockdown and the University of Maryland campus and laboratories were closed. This meant that our normal research workforce of undergraduate students is no longer available to us. While vaccines brought reduced Covid-19 risks in the first quarter of 2021, cases and risks, and restrictions increased again in the second quarter of 2021. We are moving ahead with research as best we can in the field, the Covid-19 limitations are still restricting what we are able to do. Therefore, in order to meet the project objectives, we anticipate that we will need an extra 9 months on top of the initially planned 1-year extension. What opportunities for training and professional development has the project provided? One Ph.D. student is supported in the Graduate School as a GA by the project and is gaining comprehensive experience and training in research design and implementation. He has also had the opportunity to write a research grant proposal (graduate student grant from NE SAFE), write and submit an abstract for a paper to be presented to the Agronomy Society of America national meetings, and prepare presentations for an extension field day planned for September 2021. Seven undergraduate students have assisted in conducting the research and have gained experience in everything from field experiment layout, to soil sampling, to lab analysis and data management. How have the results been disseminated to communities of interest? The early results have been disseminated via a conference panel discussion at the annual Future Harvest conference which was attended by 700 + farmers and food system professionals. 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Continue collecting operational and input expenses and income for economic analysis. Activities Planned to Meet Extension objectives: Publish at least one extension

article to disseminate information about the organic transitions study and early findings/lessons. Organize at least one farmer's field day in one of our experiment sites. The first field day has been planned for September 2021. Publish a beta version of the Grain Marketing Guide. Continue mentor-mentee activities for the two established relationships.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? On January 27th, 2020 we held the kickoff meeting for the entire project team to get to know each other better and plan strategies for accomplishing the project goals in agronomic and economic research, outreach, and farmer mentorship. On February 11th, 2020 we held our first meeting with potential farmer collaborators who were interested in hosting on-farm research or in being mentors or mentees in the organic transitions program. At this meeting, we and our collaborators from Future Harvest arranged for two mentors with many years of experience growing organic grains to work with and guide two mentees, farmers who are just embarking on the transition process with no organic farming experience. We also recruited two farmers to participate in the in-farm replicated research comparing transitional strategies. Each of these commercial farmers will compare three strategies in four replications. One of the farmers is one of the mentors and another is one of the mentees. Initially, we had another two farmers interested in doing the on-farm research, but they had to back out for logistical reasons. The fields they wanted to put through the transition was so far from their headquarters that managing, on that field, three or four different systems requiring different operations, at different times with different equipment was simply not feasible for them. Our farm management economist, Dale Johnson, set up a detailed protocol for recording all the relevant economic data (costs, hours, revenues, etc.) The managers of the four remaining research sites, two on the University of Maryland research stations and two on commercial farms, all agreed that we would begin the transition process by growing spring oats. Efforts were made in collaboration with Future Harvest and the Great Grains Alliance to find premium price markets for transitional oats. It turned out that most local specialty buyers, such as distillers, required awn-less or "naked" oats versus whole awned oats, and some of the farmers did not have the ability to clean the grain to the high degree needed for marketing at local breweries. However, all four did move ahead with the planting of spring oats as the first transition cash crop. Since this is a close-growing crop and was planted uniformly across the four field research sites it would also serve as a uniformity trial to help assess soil productivity variations as they occurred, are especially within-field variability. Replicated plots were laid out and when the oats were harvested in July individual plot yield values were recorded so that trends in spatial trends in productivity could be assessed for future use in blocking the experiment and as potential covariates for future analysis. At one of the sites, these oat yield data allowed us to rearrange the treatment plots for much better within-block homogeneity. Simultaneously with the planting of spring oats, we conducted a preliminary trial of a new organically-approved herbicide that is based on eugenol, an essential oil of the clove plant. We conducted this trial to determine whether this material could terminate cover crops to facilitate no-till planting in organic transition systems. The results of this preliminary trial showed that the herbicide (brand named Weed Slayer™) was highly effective against grassy weeds, but only partially effective against broadleaf species, such as vetch and clover, used for cover cropping. In mid-July, the harvest of spring oats at 3 sites was immediately followed by laying out the replicated treatment plots and planting a multi-species warm-season cover crop, including Sunn Hemp, Cowpeas and Soybeans, to start building soil health, organic matter and nitrogen in treatment #3 (maximum cover cropping, minimum disturbance). At the fourth site, the oat harvest was delayed until mid-August so the summer cover crop was omitted. At two sites treatment #4 (perennial alfalfa-grass hay) will be established in late August using mowing and a minimum of tillage to control weeds. Extension Accomplishments Future Harvest (FH) promised to match three farmers new organic farming with three farmer consultants well versed in the process of converting from conventional to organic grain production. Additionally, FH committed to running 2 workshops on organic grain transition (one on each side of the Bay). Thus far, Future Harvest has made two of the three mentor/mentee matches. While a number of farmers expressed interest in growing organic grain, most have lingering questions about finances and profitability, including about high transition costs as farmers may have to purchase new equipment and the uncertainty finding buyers willing to pay a premium for transitional grains. **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 No publications reported this period.

2020/09 TO 2021/08 What was accomplished under these goals? Field Research experiments have been established at four locations, two on experiment stations and two on commercial farms. The four treatments are conceptually defined and are shaped by adaptive research decisions made in collaboration with the two commercial farmers and two research farm managers. The experiment consists of three or four treatments (depending on the site) along a continuum of tillage intensity, cover crop optimization, and purchased inputs to be compared during the three-year transition period are (starting with spring of 2020) and concluding with a certified

organic corn crop in summer 2023. The treatments are Traditional full-tillage, full input, organic oat-corn-soybean-corn, with simple cover crops and input substitution. Reduced-till, moderate input, oat-corn-soybean-corn with high-biomass, early-planted cover crops terminated by roller-crimping. Minimum-till, minimum input, oat-corn-soybean-corn rotation with early-planted, precision-zoned high biomass diverse cover crops terminated by roller-crimping. Perennial alfalfa-grass hay, untilled, implemented in experiment stations only. Limed and fertilized with, manure and rock powders. Due to the lack of hay-making equipment owned by the commercial farmers or available locally to borrow or rent, the fourth treatment was established only on the two experiment stations. Field Research Accomplishments (August 2020-July 2021)

August, 2020 - Planted summer cover crops in Trt 3 at CMREC, LESREC, and Cooper sites - Baseline soil sampling at CMREC, Cooper and LESREC sites - Calculation of bulk density of baseline soil samples - Subsampling and storage of baseline soil samples at -20 degrees C for later DNA analysis, rest of the samples were air dried and processed for further analysis

September, 2020 - Biomass sampling of summer cover crops in Trt 3 from three locations per plot before termination, the samples were dried, taken dry weight, ground and stored. - Termination of summer cover crops in CMREC, LESREC, and Cooper sites. - Planting of cover crops in Trt 1, 2 and 3 at CMREC, Cooper and LESREC sites. - Planting of alfalfa-grass in Trt 4 at CMREC and LESREC.

October, 2020 - Baseline soil sampling at Cavanaugh's site. - Planting of cover crops in Trt 1, 2 and 3 at Cavanaugh's site.

November, 2020 - CANOPEO analysis of cover crop establishment at CMREC, LESREC, and Cavanaugh's site

December, 2020 - Biomass collection of cover crop samples, the samples were dried, taken dry weight, ground and stored.

January, 2021 - Collection of operations, inputs, expenses details from all four sites - Meeting with the organic transitions team for a discussion of accomplishments, issues, challenges, and future plans - Installation of soil temperature, moisture, and EC dataloggers at Cooper (2 loggers and 12 sensors) and LESREC (3 loggers and 18 sensors) - Attended Future Harvest's 22nd annual conference at which three project participants and the PI presented preliminary results and lessons learned at a panel discussion.

February, 2021 - Installation of soil temperature, moisture, and EC dataloggers CMREC (4 loggers and 24 sensors)

March, 2021 - Attended soil health innovations conference. Adaptive management discussions among researchers and four farm managers took place to develop specific practices for the four transition strategies adapted to the conditions at each site.

April, 2021 - Installation of soil temperature, moisture, and EC dataloggers at Cavanaugh's (2 loggers and 12 sensors) - Submission of a research grant to NE-SARE titled "How the transition to Organic Grain Effects Biological Indicators of Soil Health" to leverage additional funds to allow exploration of the transition treatments' effects on soil metagenomics.

May, 2021 - Collection of cover crop biomass samples from all four sites. The samples were dried, taken dry weight, ground, and stored. - Organic Termination of cover crops in Trt 1, 2 and 3 at CMREC, Cooper and LESREC by tillage, mowing, and/or roller-crimping. Precision bio-strip till cover crop was highly successful LESREC. - Harvesting of hay from trt 4 at LESREC - Planting of Blue River organic corn hybrid in Trt 1, 2, and 3 (no-till) at CMREC, Cooper and LESREC sites

June, 2021 - Planting of Blue River corn hybrid in Trt 1, 2 and 3 (no-til) at Cavanaugh's site - Assessment of corn establishment at all four sites - LECO analysis of cover crop samples for measuring percentage of C and N - Soil sampling for Pre-Sidedress Nitrate test (PSNT) analysis at V5 stage of corn at CMREC, LESREC and Cooper sites, accompanied by rapid nitrate extraction and analysis using a Horiba potentiometric ion-selective Nitrate Meter.

July, 2021 - Soil sampling for Pre-sidedress Nitrate test (PSNT) analysis at V5 stage of corn at Cavanaugh's site, PSNT analysis using Horiba Nitrate Meter - Assessment of weeds in all treatments - Harvesting of Hay from Trt 4 at CMREC

Future Harvest and Extension Accomplishments. Future Harvest committed to matching three farmers new to the transition process with three farmer consultants well versed in the process of converting from conventional to organic grain production. Additionally, Future Harvest committed to running 2 workshops (one on Delmarva and one on the western side of the Chesapeake Bay) on organic grain transition. Thus far, Future Harvest has made two of the three mentor-mentee matches proposed. It turned out that while a number of farmers were interested in growing organic grain, few were willing to make the switch from conventional to organic due to lingering questions about the finances of the process. This is a valid and realistic concern given the narrow profit margins farmers face, the different equipment needs from conventional to organic (hence possibly high transition costs as farmers may have to purchase new equipment), the uncertainty of their ability to find buyers willing to pay a premium for their transitional grains during the 3 yr transition process, and general questions about the market conditions for organic grain locally. Future Harvest partnered with Extension to run a workshop called "Ask A Farmer -- Organic Grain, Does It Pay?". This workshop consisted of a panel discussion and Q&A space to allow farmers to identify whether a transition is a good fit for them and if so to get connected into our Future Harvest's Great Grains Project. Farmers Aaron Cooper of Cutfresh Organics and Steve Krazowski of Mason's Heritage Farm were the farmer panelists answering conventional growers' questions about organics. Extension agent Shannon Dill was on the panel to answer financial questions and extension agent Sarah Hirsh both facilitated the session and answered questions about soil health. The second Future Harvest workshop is being organized in partnership with the Common Grain Alliance. As the Organic Transitions project research moved forward in 2020, the research team decided to integrate a small grain into the crop rotation (oats). As we searched for premium buyers of these oats, we've learned much along the way. We learned that the brewer community is interested in

purchasing local oats, but that they have very specific types and quantities they need. For this second Future Harvest workshop, we will bring a local brewer together with a local farmer to model the conversation about requirements and preferences in the buying and selling process so that members of the research, farming, and brewing communities know how to meet each other's needs. Lastly, Future Harvest has been working on creating a directory of buyers for local, transitional grain. This process is well underway and continues with the assistance of three University of Maryland undergraduate students working for the project. **PUBLICATIONS (not previously reported):** 2020/09 TO 2021/08 No publications reported this period. ** **

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Linking Plant Traits with Soil Health to Determine Optimal Cover Crop Mixtures on Organic Farms

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NON-TECHNICAL SUMMARY

Cover crops, or non-harvested crops, are essential components of organic crop rotations. Organic farmers plant cover crops in the legume family to supply nitrogen to fields, and are increasingly experimenting with cover crop mixtures to provide multiple ecosystem services at once. Key services of interest to farmers include retaining soil nitrogen and phosphorus, building soil organic matter, and controlling weeds. There is a critical need to determine how the diversity of plant traits in multi-species cover crop mixtures, and resulting ecosystem services, vary across environmental gradients. Our multi-institution research and extension project will use novel approaches, including a participatory experiment on 15 working farms, to address several knowledge gaps for optimal mixture performance. First, we will determine mixture performance along a soil health gradient resulting from differences in farm management history and soil type. Second, we will identify relationships between plant functional trait diversity in cover crop mixtures and ecosystem services. Third, we will quantify legume nitrogen fixation, phosphorus cycling, root biomass, and weed suppression on farms to support the success of diversified, organic cropping systems. Finally, we will generate practical management recommendations for organic and transitioning farmers, including an economic assessment of mixtures, which will support broader implementation of cover crop mixtures in Michigan and beyond. Knowledge of cover crop mixtures that improve ecological nutrient management on farms could be extended to address broader conservation goals for water quality. Results will also inform improvements to an online cover crop decision tool, and will be shared widely through outreach activities including extension bulletins, presentations at farmer meetings, field days, workshops, and engagement with policy communities.

OBJECTIVES

The major goal of this project is to determine the optimal management of cover crop mixtures on transitioning and organic grain farms to enhance multiple ecosystem services. In Michigan, grain farms span tremendous diversity in soil types, and the state has one of the largest contiguous groups of organic grain farmers in the U.S., primarily located in the eastern "thumb" region (Figure 1). These factors provide a unique opportunity to evaluate cover crop mixtures on Michigan farms as an economically feasible and practical approach to diversifying grain rotations. Agricultural systems tend to have lower biodiversity than unmanaged ecosystems, with most plant species selected by farmers. Thus, instead of overall species diversity, functional diversity - the diversity of plant functional traits, which are characteristics of plants that determine their effects on, or responses to, the

environment - is a crucial predictor of ecosystem services including productivity, and multifunctionality (i.e., the provisioning of multiple ecosystem services at once). For example, nitrogen (N) fixation by legumes is a functional trait that supplies N to fields. Cover crop mixtures that include legumes may provide a nitrogen (N) source along with other functions such as enhancing phosphorus (P) availability to primary crops, building soil organic matter, and improving water infiltration, all of which are major goals for land transitioning to certified organic production. This project has three overarching research and extension objectives: (1) In an on-farm experiment, identify soil properties that predict ecosystem services and multifunctionality of cover crop mixtures. Question 1.1. How do soil properties influence the performance of cover crop mixtures? Question 1.2. Which soil properties predict legume biomass and BNF inputs in mixtures across farms? (2) Identify soil properties that predict variation in cover crop functional traits. Question 2.1. Which soil parameters best explain plant trait variation (leaf N and P content, root:shoot, maximum height, and specific leaf area) across farms spanning a gradient of soil health levels and N availability from soil organic matter? (3) Provide practical recommendations for organic and transitioning farmers on how to best design and manage cover crop mixtures to maximize ecosystem services, including consideration of economic returns.

APPROACH

Objective 1: We will identify farms located on two different soil types in Michigan, with the expectation that each soil type would include a cluster of farms spanning contrasting management histories and thus soil health. Participating farmers will be compensated for their time and land. The research will require that they have two fields to use for this study (one field in each year). A maximum of 1.2 ha (3 acres) of each field will be used to grow the cover crops. Each field will be planted in a small grain crop of their choice (two fields total per farm x 15 farms). We will work with farmers to match the size of the study area to their farm equipment, particularly grain drills for seeding cover crops and harvesting equipment. We will conduct in-depth interviews to document farmers' goals for cover crop mixtures as well as their farm management histories. For all of the fields in the study we will collect detailed information about crop rotations, tillage, field inputs, and weed management practices. We will assess background environmental conditions by collecting weather information (growing degree days, precipitation, etc.) from the weather station closest to each farm. We will fully characterize the soils of the two study fields per farm (one field per year) with a baseline sample to 20-cm depth taken in spring, after spring grain planting, or at the start of spring growth for winter grains. We will measure bulk density at sampling. A sub-sample will be analyzed at the A&L Great Lakes commercial laboratory for a standard soil test including macro- and micro-nutrients, soil texture, and pH. Subsamples will be analyzed in our respective labs for soil moisture, total organic C and total N (dry combustion), soil inorganic N (KCl extraction), soil aggregate stability, potentially mineralizable N, and short-term C-mineralization. We will also measure free and protected particulate organic matter (POM), and POM C and N, another biological indicator of soil health. This suite of physical, chemical, and biological measures will allow us to determine how background soil conditions reflecting soil type and management history affect cover crop establishment, composition, and performance. In August of each study year, following small grain harvest, farmers will plant the cover crop plots on the selected field in a randomized complete block design with assistance from our research team. We will provide farmers with certified organic seed for all treatments. Plots will be arranged to accommodate farm equipment. Farmers will use their own grain drills wherever possible, with our team's assistance to mark the plots and clean the drill between treatments. The treatments will include two mixtures of 3-4 functionally diverse species, a legume monoculture treatment, and a cereal rye monoculture treatment as a benchmark, because this is the most commonly planted winter cover crop in Michigan. Both cool-season cover crop mixtures will include three plant families (grass, legume, brassica), and two plant functional groups (legume, non-legume), designed to harness complementary plant traits. Before planting the on-farm experiment, we will hold a meeting with partnering farmers to discuss the process and expected outcomes of the experiment for their feedback on the proposed mixtures. We will determine an appropriate seeding rate for each treatment based on outcomes of the field station experiment, principles of plant functional ecology, and participating farmer input. Examples of three-species mixtures that may meet the selection criteria are: cereal rye, Austrian winter pea, and oriental mustard, or annual ryegrass, crimson clover, and daikon radish. All plots will be sampled at two time points, in the fall before cover crop dormancy, and in May before cover crop incorporation and cash crop planting, to measure cover crop establishment and mixture composition, total aboveground biomass (from four replicate 0.25m² quadrats), and biomass %N and %P (to calculate nutrient retention in biomass), BNF (N supply), winter survival, and weed suppression. We will measure BNF at early flowering using the ¹⁵N natural abundance method with rye monocultures as a reference plant. We will measure weed suppression by comparing total weed biomass in the mixtures and legume monoculture versus the cereal rye, which is known to be a powerful weed suppressor. The study fields will be planted to the same cash crop following the cover crop (where feasible for farmers) to assess impacts on yield. We will determine field-scale N use efficiency with N mass balances, using the two-year results estimating BNF inputs on each farm to improve the 5-year N balances calculated from farmer interviews. We will test for treatment

differences for individual ecosystem services using hierarchical linear mixed-effects models with treatment as a fixed effect, and block nested in farm as a random effect. We will then use linear regression to identify variables that predict services (e.g., biomass, N fixation, N and P retention, weed suppression, etc.). The regression models will include soil health measures and other properties from soil analyses; environmental conditions; and management variables from the farmer interviews as predictor variables. Finally, we will use structural equation modeling to estimate causal relationships between the set of farm management variables, N mass balance, environmental variables (e.g., soil texture, temperature, moisture), soil health measures, and the multiple ecosystem services that we measure.

Objective 2: Using data from baseline soil sampling on farms, and from management interviews conducted with all farmers in Objective 1, we will select 7-9 farm fields on similar soil types that span a management-induced fertility gradient. On these fields, in addition to the methods detailed in Objective 1, we will measure several plant traits that are expected to vary due to management practices. We will measure root:shoot of all cover crop species; leaf C:N:P (by separating leaves from stems), maximum plant height, and specific leaf area (SLA) as the ratio of leaf surface area (cm²) divided by leaf dry mass (g).

Objective 3: With the farmer interview data we will calculate costs and returns for planting cover crop mixtures within organic and transitioning management systems. We anticipate starting the assessment with a basic analysis of costs and returns, including marginal costs of cover cropping and cash crop yield and price for both organic and transitioning farmers. Once we have the data on ecosystem services provided by different treatments, such as N supply from legume N fixation, or estimates of net C input to soil, we will expand the approach to include potential added returns from payments for ecosystem services schemes. To estimate these added returns, we will use benchmark prices from existing programs or other incentives for conservation available in the Great Lakes region such as "avoided N" or C sequestration. In-depth evaluation of the proposed research and extension activities will include assessing farmer impact, such as levels of participation, and anticipated and actual (after 6 months) inclusion of cover crops in farm management by participants of field days and workshops. We will compare pre- and post-project surveys conducted with participating farmers to assess change in interest and use of cover crops.

Progress 09/01/22 to 08/31/23

Outputs

Target Audience: Our primary target audience is a group of organic and transitioning farmers who partnered with us on this on-farm research. Their farms are located in two regions of Michigan: the "Thumb" and southwestern regions. Fourteen farmers participated in the first year of the on-farm experiment. In the second year of the experiment, we worked with a group of 13 farmers. Our project results are also relevant to the broader group of organic and transitioning grain farmers in Michigan and the Midwest. Midwestern farmers who grow small grains have a unique window for planting cover crops following small grain harvest. This gap in crop production while the weather is still warm offers an opportunity to plant many cover crop types and mixtures. Michigan's organic farmers are interested in opportunities to test cover crop mixtures that can enhance soil quality and diversify their crop rotations. We have also shared results with organic vegetable farmers in the Great Lakes region, and with a wider group of agricultural professionals in Michigan through facilitated Roundtable Discussions with stakeholders including members of government agencies, non-profit organizations, the private sector, extension educators, and other researchers.

Changes/Problems: Despite the large logistical challenges of conducting research during the Covid-19 pandemic, we planted the experiment as scheduled on 14 farms in Michigan. However, aspects of all objectives were affected and/or delayed due to Covid-19. For instance, we exceeded transportation costs included in our budget because researchers had to drive to field sites in individual vehicles to meet the safety requirements. We also had extra labor costs because it took extra time to do standard field and lab activities due to extra safety requirements and restrictions on the number of people who could work in a given space. We had to delay the in-person farmer interviews, which led to lower levels of participation than planned, and in-person outreach activities were delayed until 2022-2023. We also had one methods-related challenge, which is common with on-farm research, where we were unable to use the same drill for planting cover crop treatments in both regions due to the cost of moving heavy equipment long distances. While both planters were seed drills, we were only able to control for seeding depth with one of them, which means there may be slight differences in planting depth across the two regions. However, we noted similar cover crop establishment and biomass in both regions, and this did not seem to have a detectable effect on our results. We resolved this issue for the second year of the study and used the same drill in both regions.

What opportunities for training and professional development has the project provided? Training activities for this project period were focused on one-on-one work with mentors for the two PhD students on the project. Both PhD students have a dissertation chapter that focuses on a sub-objective of this project. The PhD students are currently completing their data analysis and have shared preliminary results with scientific and practitioner communities. To complete the final stages of lab work we involved and trained a group of six undergraduate students who gained experience in laboratory techniques, specifically soil health analyses and preparation of samples for stable isotope analysis. How have the results been disseminated to communities of interest? We spent several months writing detailed, personalized farmer reports for each participant in the study. The well-designed, 10-page reports summarized the study goals and methods, key project takeaways, and specific results. Results were organized into sections on cover crop biomass, cover crop N and P recycling in aboveground biomass and roots, new N supplied by crimson clover N fixation, the performance of the "wildcard" (farmer-

selected) mixtures across farms, and definitions of all soil health indicators with raw values and soil health scores for each field. For each of these outcomes, farmers received data on their own fields, as well as average values for all farms (either in the whole study, or in their region). In the final reporting period we also presented project findings to farmers in this study, other farmers in Michigan and the Great Lakes region, and other agricultural stakeholders through presentations at farmer conferences (Underground Innovations, Marbleseed, Conservation Tillage and Technology Conference, and Great Lakes EXPO), at field days (in Clayton, MI, and in Berrien Center, MI) and in a Roundtable Discussion with diverse stakeholders from the agricultural community. The conversation began with a presentation of results from the research by the PD, followed by a facilitated discussion to receive feedback on the data, to help interpret and provide context for the results, and to generate new ideas for participatory research. Key outcomes shared with these communities: Our project generated new ecological knowledge of drivers of variation in the functional traits and ecosystem functions provided by cover crop mixtures on working organic grain farms. Our research improved quantification of cover crop biomass production, N and P recycling, and legume N supply, across a soil health gradient, which can improve site-specific cover crop and nutrient management recommendations for organic farmers to enhance sustainability and reduce input costs (e.g., compost, manure). These findings also suggest the need for more support (cost-share, knowledge) for growers in the early phases of adoption to help them reach a threshold of strong performance and the positive feedback between biomass and cover crop functions. Based on the experiment, interviews, and feedback from participants, our results suggest that there are biophysical reasons why cover crops may not perform well in the early stages of transitioning lower fertility soils to diversified, organic crop production. When first adopting cover crops, our results suggest it may be most effective to use single species cover crops (of different functional groups, depending on management goals) at different points in rotation, while transitioning to more diverse cover crop mixtures once soil health has begun to improve. On average, the 4-way mixture had higher biomass production, P uptake, and weed suppression than both sole cover crops in both years of the study. Our results also show that for farmers with high soil health, especially strong performance of indicators of soil N cycling (such as PMN and POM), legumes will not be as competitive or fix as much N, suggesting that farmers can focus on grass and brassica cover crops to retain and recycle existing soil N. Finally, results for the non-legume cover crops show a positive feedback loop where biomass increased with indicators of soil health, suggesting that ecosystem services from cover crops will increase with repeated use. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported Impacts What was accomplished under these goals? Our multi-institution project conducted participatory research on farms to understand cover crop mixture performance along a soil health gradient; identified novel relationships between functional trait diversity in mixtures and multiple ecosystem services; and generated management recommendations for organic farmers, including an economic assessment of cover crops, which will support broader use of cover crop mixtures. Results were shared through detailed reports for each participant, and extension presentations at farmer conferences, field days, and engagement with policy communities. By identifying mixtures that improve ecological nutrient management on farms, our results inform how to realize regional goals for water quality and climate-smart agriculture. Objective 1: In an on-farm experiment, identify soil properties that predict ecosystem services and multifunctionality of cover crop mixtures. Major activities and data We analyzed plant samples for total phosphorus (P). We removed carbonates from high pH soils to determine soil organic C (SOC) concentrations and stocks. We used scoring functions to calculate soil health scores by field. We received stable isotope results and C and N concentrations for particulate organic matter (POM) samples. Results Ecosystem services: The mixture had the highest multifunctionality (i.e., providing multiple services at once), while sole clover provided the greatest N supply. Across farms, in both years the mixture had highest biomass for combined fall and spring samples (5638 kg/ha in year 1 and 4793 in year 2), while rye was the lowest (3172 and 3010 kg/ha; $p < 0.0001$). Year 2 had a cooler, wetter spring, and most services differed by year. In mixed models (by year) with cover crop treatment and region, their interaction, and block nested in farm as a random effect, there was a significant region by treatment interaction for all services. In spring of year 1, the mix and sole clover had similar shoot biomass (~ 3150 kg/ha) in the southwest (SW), while in the thumb, the mix had highest biomass (3921 kg/ha). In year 2, the mix had the highest spring biomass in the SW (2988; $p < 0.0001$), while in the thumb, sole clover (2219) was higher than mix (1573). The cover crop treatments assimilated 35.7-94.1 kg N/ha with highest mean N retention in the mix and clover, while rye had lower shoot N ($p < 0.0001$). Mean shoot P uptake ranged from 5.3 kg P/ha (rye and clover in year 2) to 15.3 kg P/ha in the mix in year 1. Overall, the mix had the highest P uptake ($p < 0.0001$). The SW had higher weed pressure; weed biomass was highest in clover, followed by rye, and then mix. In the Thumb, weed biomass was lowest in the mix in year 1, while in year 2, rye and mix did not differ, and both had lower weed biomass than clover. Clover N fixation differed by year ($p < 0.0001$) and treatment ($p < 0.0001$). In year 1, sole clover supplied a mean of 56.6 kg N/ha vs. 25 kg N/ha for clover in mixture. Fixed N was strongly related to clover biomass ($p < 0.0001$). In year 2, when biomass was lower, clover N fixation means were 25.5 and 15.7 kg N/ha for sole and mix treatments. Soil health: There was a wide soil health gradient across farms, with mean normalized soil health scores (scale of 0-100) ranging from 36-79 in the SW and from 37-68 in the Thumb. The regions had contrasts in soil health that affected cover crop outcomes. Thumb soils had higher

%clay, CEC, pH, SOC stocks to 20 cm (mean of 42.74 Mg C/ha vs. 31 in the SW), and indicators that reflect stable soil C (e.g., POX-C, higher C:N). SW soils had lower %clay, pH, and SOC, but had higher indicators of biological soil health that reflect microbial activity and nutrient cycling (e.g., soil inorganic N, PMN, PMC, and C and N in free and protected POM). Soil health and cover crop functions: We are still completing this analysis. Mixed effects regression models showed that clover biomass was negatively related to indicators of soil N availability depending on year, including inorganic N ($p=0.026$) and POM N ($p=0.001$), while weed biomass increased with these indicators. These relationships were not significant for clover in mixture, where clover was competing with other species for soil N. Indicators of soil inorganic N were also negatively related to clover N fixation rate (kg N/ha) in monoculture and mixture, and to the % of clover N from the atmosphere. Cover crop P uptake was positively related to indicators including Bray-1 P, clay, CEC, pH, and POM. Rye biomass increased with soil health, with strong positive relationships with PMC ($p<0.0001$), PMN ($p=0.013$), and Bray-1 P ($p=0.001$) in year 1, and with POX-C ($p=0.041$), Bray P (0.04) and CEC ($p=0.0001$) in year 2. Objective 2: Identify soil properties that predict variation in cover crop functional traits. Major activities and data To complete the cover crop functional trait dataset, we digested and analyzed 625 leaf and root samples for %P. We compiled a plot-level dataset of nine cover crop functional traits and twenty soil properties for 13 fields (6 in year 1, 7 in year 2) in the Thumb region. Summary statistics and results PCA showed that rye, clover, and dwarf essex rapeseed occupied distinct functional trait spaces. This suggests interspecific niche complementarity when grown together in mixture, which may enhance overall resource-use efficiency and agroecosystem multifunctionality. We also found intraspecific trait variation in response to the soil health gradient and cover crop treatment, using mixed effect models with treatment, year, and their interaction as fixed effects, and block nested in field nested in farm as random effects. Aboveground traits for rye and clover (maximum height, SLA, leaf %N, leaf %P, shoot C:N) differed significantly between monoculture and mixture treatments, indicating that interactions between species in mixture induced trait plasticity. Across the soil health gradient, clover and rye traits responded strongly to protected POM N and soil P, while free POM N and PMN were better predicted variation in dwarf essex traits. For clover, leaf %P, root %P, and root %N all increased, while shoot and root C:N decreased, in response to soil P availability. There were positive relationships between protected POM N and clover leaf %P, SLA, and root:shoot, but negative relationships between pH and root:shoot, leaf %N, and root %N. For rye, SLA, root %P, and root:shoot all increased with protected POM N. Rye root %P and root:shoot also increased with soil P, whereas SLA and shoot C:N decreased with soil P. Several acquisitive traits for dwarf essex, including SLA, leaf %N, and root %P, responded positively to increasing free POM N. And dwarf essex root %N increased with PMN. Objective 3: Provide practical recommendations on how to best design and manage cover crop mixtures to maximize ecosystem services, including consideration of economic returns. Major activities We analyzed data from the interviews with 6 farmer participants on cost and return estimates for our treatments and shared findings and recommendations widely. Results Detailed economic interview data show the predicted net change in income from adopting the cover crops we tested relative to baseline cover crop practices varied widely. Net change in income relative to baseline ranged from $-\$257.35$ to $\$28.71$ /acre for cereal rye; from $-\$31.75$ to $\$115.90$ /acre for crimson clover; from $-\$34.91$ to $\$106.35$ /acre for the 4-way mix; and from $-\$62.89$ to $\$134.12$ /acre for the farmer-selected mix. The farm with a large estimated decrease in income with rye typically plants a legume-based mixture; the reduction was based on estimated extra compost costs without legumes as a N source. To standardize across farms, we calculated the change in net income per acre relative to rye, and found that the 4-way mix and wildcard provided increases of $\$65.86$ /acre and $\$65.29$ /acre relative to rye, while clover had a small increase of $\$2.36$ /acre. Key outcomes are described below. Publications Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Ward, M., Herrick (Sutton), E. and J. Blesh. 2022. Using observational and experimental approaches to understand cover crop performance. Poster presentation. Undergraduate Research Opportunity Program (UROP) Symposium, University of Michigan, Ann Arbor, MI, April 20. Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Herrick (Sutton), E., and J. Blesh. 2022. Belowground cover crop traits and functions across a soil health gradient. Oral presentation. Ecological Society of America Annual Meeting, Montreal, Canada, Aug. 15. Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: KC, R., and S. S. Snapp. 2022. Nitrogen Synchrony with Cover Crops in Organic Corn Systems. Poster presentation. ASA, CSSA, SSSA International Annual Meeting, Baltimore, MD, Nov. 7. Progress 09/01/19 to 08/31/23 Outputs Target Audience: Our primary target audience is a group of organic and transitioning farmers who partnered with us on this on-farm research. Their farms are located in two regions of Michigan: the "Thumb" and southwestern regions. Fourteen farmers participated in the first year of the on-farm experiment. In the second year of the experiment, we worked with a group of 13 farmers. Our project results are also relevant to the broader group of organic and transitioning grain farmers in Michigan and the Midwest. Midwestern farmers who grow small grains have a unique window for planting cover crops following small grain harvest. This gap in crop production while the weather is still warm offers an opportunity to plant many cover crop types and mixtures. Michigan's organic farmers are interested in opportunities to test cover crop mixtures that can enhance soil quality and diversify their crop rotations. We have also shared results with organic vegetable farmers in the Great Lakes region, and with a

wider group of agricultural professionals in Michigan through facilitated Roundtable Discussions with stakeholders including members of government agencies, non-profit organizations, the private sector, extension educators, and other researchers. Changes/Problems: Despite the large logistical challenges of conducting research during the Covid-19 pandemic, we planted the experiment as scheduled on 14 farms in Michigan. However, aspects of all objectives were affected and/or delayed due to Covid-19. For instance, we exceeded transportation costs included in our budget because researchers had to drive to field sites in individual vehicles to meet the safety requirements. We also had extra labor costs because it took extra time to do standard field and lab activities due to extra safety requirements and restrictions on the number of people who could work in a given space. We had to delay the in-person farmer interviews, which led to lower levels of participation than planned, and in-person outreach activities were delayed until 2022-2023. We also had one methods-related challenge, which is common with on-farm research, where we were unable to use the same drill for planting cover crop treatments in both regions due to the cost of moving heavy equipment long distances. While both planters were seed drills, we were only able to control for seeding depth with one of them, which means there may be slight differences in planting depth across the two regions. However, we noted similar cover crop establishment and biomass in both regions, and this did not seem to have a detectable effect on our results. We resolved this issue for the second year of the study and used the same drill in both regions. What opportunities for training and professional development has the project provided? Training activities for this project period were focused on one-on-one work with mentors for the two PhD students on the project. Both PhD students have a dissertation chapter that focuses on a sub-objective of this project. The PhD students are currently completing their data analysis and have shared preliminary results with scientific and practitioner communities. To complete the final stages of lab work we involved and trained a group of six undergraduate students who gained experience in laboratory techniques, specifically soil health analyses and preparation of samples for stable isotope analysis. How have the results been disseminated to communities of interest? We spent several months writing detailed, personalized farmer reports for each participant in the study. The well-designed, 10-page reports summarized the study goals and methods, key project takeaways, and specific results. Results were organized into sections on cover crop biomass, cover crop N and P recycling in aboveground biomass and roots, new N supplied by crimson clover N fixation, the performance of the "wildcard" (farmer-selected) mixtures across farms, and definitions of all soil health indicators with raw values and soil health scores for each field. For each of these outcomes, farmers received data on their own fields, as well as average values for all farms (either in the whole study, or in their region). In the final reporting period we also presented project findings to farmers in this study, other farmers in Michigan and the Great Lakes region, and other agricultural stakeholders through presentations at farmer conferences (Underground Innovations, Marbleseed, Conservation Tillage and Technology Conference, and Great Lakes EXPO), at field days (in Clayton, MI, and in Berrien Center, MI) and in a Roundtable Discussion with diverse stakeholders from the agricultural community. The conversation began with a presentation of results from the research by the PD, followed by a facilitated discussion to receive feedback on the data, to help interpret and provide context for the results, and to generate new ideas for participatory research. Key outcomes shared with these communities: Our project generated new ecological knowledge of drivers of variation in the functional traits and ecosystem functions provided by cover crop mixtures on working organic grain farms. Our research improved quantification of cover crop biomass production, N and P recycling, and legume N supply, across a soil health gradient, which can improve site-specific cover crop and nutrient management recommendations for organic farmers to enhance sustainability and reduce input costs (e.g., compost, manure). These findings also suggest the need for more support (cost-share, knowledge) for growers in the early phases of adoption to help them reach a threshold of strong performance and the positive feedback between biomass and cover crop functions. Based on the experiment, interviews, and feedback from participants, our results suggest that there are biophysical reasons why cover crops may not perform well in the early stages of transitioning lower fertility soils to diversified, organic crop production. When first adopting cover crops, our results suggest it may be most effective to use single species cover crops (of different functional groups, depending on management goals) at different points in rotation, while transitioning to more diverse cover crop mixtures once soil health has begun to improve. On average, the 4-way mixture had higher biomass production, P uptake, and weed suppression than both sole cover crops in both years of the study. Our results also show that for farmers with high soil health, especially strong performance of indicators of soil N cycling (such as PMN and POM), legumes will not be as competitive or fix as much N, suggesting that farmers can focus on grass and brassica cover crops to retain and recycle existing soil N. Finally, results for the non-legume cover crops show a positive feedback loop where biomass increased with indicators of soil health, suggesting that ecosystem services from cover crops will increase with repeated use. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported

Impacts
What was accomplished under these goals? Our multi-institution project conducted participatory research on farms to understand cover crop mixture performance along a soil health gradient; identified novel relationships between functional trait diversity in mixtures and multiple ecosystem services; and generated management recommendations for organic farmers, including an economic assessment of cover crops, which will support broader use of cover crop mixtures. Results were shared through detailed reports for each participant, and

extension presentations at farmer conferences, field days, and engagement with policy communities. By identifying mixtures that improve ecological nutrient management on farms, our results inform how to realize regional goals for water quality and climate-smart agriculture.

Objective 1: In an on-farm experiment, identify soil properties that predict ecosystem services and multifunctionality of cover crop mixtures. Major activities and data We analyzed plant samples for total phosphorus (P). We removed carbonates from high pH soils to determine soil organic C (SOC) concentrations and stocks. We used scoring functions to calculate soil health scores by field. We received stable isotope results and C and N concentrations for particulate organic matter (POM) samples. Results Ecosystem services: The mixture had the highest multifunctionality (i.e., providing multiple services at once), while sole clover provided the greatest N supply. Across farms, in both years the mixture had highest biomass for combined fall and spring samples (5638 kg/ha in year 1 and 4793 in year 2), while rye was the lowest (3172 and 3010 kg/ha; $p < 0.0001$). Year 2 had a cooler, wetter spring, and most services differed by year. In mixed models (by year) with cover crop treatment and region, their interaction, and block nested in farm as a random effect, there was a significant region by treatment interaction for all services. In spring of year 1, the mix and sole clover had similar shoot biomass (~3150 kg/ha) in the southwest (SW), while in the thumb, the mix had highest biomass (3921 kg/ha). In year 2, the mix had the highest spring biomass in the SW (2988; $p < 0.0001$), while in the thumb, sole clover (2219) was higher than mix (1573). The cover crop treatments assimilated 35.7-94.1 kg N/ha with highest mean N retention in the mix and clover, while rye had lower shoot N ($p < 0.0001$). Mean shoot P uptake ranged from 5.3 kg P/ha (rye and clover in year 2) to 15.3 kg P/ha in the mix in year 1. Overall, the mix had the highest P uptake ($p < 0.0001$). The SW had higher weed pressure; weed biomass was highest in clover, followed by rye, and then mix. In the Thumb, weed biomass was lowest in the mix in year 1, while in year 2, rye and mix did not differ, and both had lower weed biomass than clover. Clover N fixation differed by year ($p < 0.0001$) and treatment ($p < 0.0001$). In year 1, sole clover supplied a mean of 56.6 kg N/ha vs. 25 kg N/ha for clover in mixture. Fixed N was strongly related to clover biomass ($p < 0.0001$). In year 2, when biomass was lower, clover N fixation means were 25.5 and 15.7 kg N/ha for sole and mix treatments. Soil health: There was a wide soil health gradient across farms, with mean normalized soil health scores (scale of 0-100) ranging from 36-79 in the SW and from 37-68 in the Thumb. The regions had contrasts in soil health that affected cover crop outcomes. Thumb soils had higher %clay, CEC, pH, SOC stocks to 20 cm (mean of 42.74 Mg C/ha vs. 31 in the SW), and indicators that reflect stable soil C (e.g., POX-C, higher C:N). SW soils had lower %clay, pH, and SOC, but had higher indicators of biological soil health that reflect microbial activity and nutrient cycling (e.g., soil inorganic N, PMN, PMC, and C and N in free and protected POM). Soil health and cover crop functions: We are still completing this analysis. Mixed effects regression models showed that clover biomass was negatively related to indicators of soil N availability depending on year, including inorganic N ($p = 0.026$) and POM N ($p = 0.001$), while weed biomass increased with these indicators. These relationships were not significant for clover in mixture, where clover was competing with other species for soil N. Indicators of soil inorganic N were also negatively related to clover N fixation rate (kg N/ha) in monoculture and mixture, and to the % of clover N from the atmosphere. Cover crop P uptake was positively related to indicators including Bray-1 P, clay, CEC, pH, and POM. Rye biomass increased with soil health, with strong positive relationships with PMC ($p < 0.0001$), PMN ($p = 0.013$), and Bray-1 P ($p = 0.001$) in year 1, and with POX-C ($p = 0.041$), Bray P (0.04) and CEC ($p = 0.0001$) in year 2.

Objective 2: Identify soil properties that predict variation in cover crop functional traits. Major activities and data To complete the cover crop functional trait dataset, we digested and analyzed 625 leaf and root samples for %P. We compiled a plot-level dataset of nine cover crop functional traits and twenty soil properties for 13 fields (6 in year 1, 7 in year 2) in the Thumb region. Summary statistics and results PCA showed that rye, clover, and dwarf essex rapeseed occupied distinct functional trait spaces. This suggests interspecific niche complementarity when grown together in mixture, which may enhance overall resource-use efficiency and agroecosystem multifunctionality. We also found intraspecific trait variation in response to the soil health gradient and cover crop treatment, using mixed effect models with treatment, year, and their interaction as fixed effects, and block nested in field nested in farm as random effects. Aboveground traits for rye and clover (maximum height, SLA, leaf %N, leaf %P, shoot C:N) differed significantly between monoculture and mixture treatments, indicating that interactions between species in mixture induced trait plasticity. Across the soil health gradient, clover and rye traits responded strongly to protected POM N and soil P, while free POM N and PMN were better predicted variation in dwarf essex traits. For clover, leaf %P, root %P, and root %N all increased, while shoot and root C:N decreased, in response to soil P availability. There were positive relationships between protected POM N and clover leaf %P, SLA, and root:shoot, but negative relationships between pH and root:shoot, leaf %N, and root %N. For rye, SLA, root %P, and root:shoot all increased with protected POM N. Rye root %P and root:shoot also increased with soil P, whereas SLA and shoot C:N decreased with soil P. Several acquisitive traits for dwarf essex, including SLA, leaf %N, and root %P, responded positively to increasing free POM N. And dwarf essex root %N increased with PMN.

Objective 3: Provide practical recommendations on how to best design and manage cover crop mixtures to maximize ecosystem services, including consideration of economic returns. Major activities We analyzed data from the interviews with 6 farmer participants on cost and return estimates for our treatments and shared findings and recommendations widely. Results Detailed economic interview data show the

predicted net change in income from adopting the cover crops we tested relative to baseline cover crop practices varied widely. Net change in income relative to baseline ranged from -\$257.35 to \$28.71/acre for cereal rye; from -\$31.75 to \$115.90/acre for crimson clover; from -\$34.91 to \$106.35/acre for the 4-way mix; and from -\$62.89 to \$134.12/acre for the farmer-selected mix. The farm with a large estimated decrease in income with rye typically plants a legume-based mixture; the reduction was based on estimated extra compost costs without legumes as a N source. To standardize across farms, we calculated the change in net income per acre relative to rye, and found that the 4-way mix and wildcard provided increases of \$65.86/acre and \$65.29/acre relative to rye, while clover had a small increase of \$2.36/acre. Key outcomes are described below.

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Changes/Problems: We have no major challenges or problems to report from this period. It is worth noting that we are still running behind schedule on several project activities as a result of delays early in the project due to Covid-19. However, we expect to complete most project activities in the next reporting period. What opportunities for training and professional development has the project provided? Training activities for this project period were focused on one-on-one work with mentors for the two PhD students and one master's student on the project. Both PhD students have a dissertation chapter that focuses on a sub-objective of this project. One of the PhD students developed his dissertation proposal and presented preliminary findings at an international meeting. The other PhD student is using project data to advance her analytical skills in a multivariate statistics course. To complete field and lab work for the project we involved and trained a larger group of students: this included 5 Master's students and 11 undergraduate students from the University of Michigan and 2 undergraduates and a graduate student from Michigan State University. Many of these students participated for a short period (e.g., several days during major field campaigns) in a volunteer capacity to gain research experience and training. Students gained experience in soil sampling and analysis techniques, as well as plant biomass and functional trait assessments. How have the results been disseminated to communities of interest? To share results with the scientific community, one of the PhD students on the project presented a poster with preliminary results on agroecosystem nitrogen dynamics across a subset of the farm sites at the 2022 ASA, CSSA, SSSA International Annual Meeting held from November 6-9, 2022 in Baltimore, MD. We also hosted a field day for participating farmers and the research team on an organic grain farm in the Thumb region. The half-day meeting was attended by most of the farm partners in that region, in addition to several other farmers who are not formally collaborating on the experiment. During the meeting, the research team (the PD and both PhD students) presented results to date, including sharing an individual handout with key results for each participating farm. Co-PD Morrone facilitated a broader discussion of farmers' experiences with the research process, knowledge exchange about cover crop management and performance, and needs and interests related to on-farm research now and in the future. We also explained what we plan to include in the final project reports to farmers, and solicited feedback on any additional information and management recommendations to include. We are preparing these individual farm reports in winter 2022-23. What do you plan to do during the next reporting period to accomplish the goals? Objective 1: We will complete the final laboratory analyses for this objective by the end of December 2022. This includes analysis of aboveground biomass samples collected from field 2 for nitrogen and delta 15N (at the UC Davis Stable Isotope Facility), which will be used to quantify nitrogen supply from clover nitrogen fixation. We are also still analyzing anion resin bags for potentially leachable nitrogen from the site at Kellogg Biological Station. We will then compile data from both experimental years to address our two main research questions for this objective: Question 1.1. How do soil properties influence the performance of cover crop mixtures? And, Question 1.2. Which soil properties predict legume biomass and legume N fixation inputs in mixtures across farms? We are currently analyzing data from the management interviews we conducted with each farmer to quantify N and P mass balances and identify other management factors that can be used as

predictors in statistical models. We will test for treatment differences for individual ecosystem services using hierarchical linear mixed-effects models with treatment and year as fixed effects, and block nested in farm as a random effect. We will then use linear regression to identify variables that predict services (e.g., biomass, N fixation, N and P retention, weed suppression, etc.). The regression models will include soil health measures and other properties from soil analyses; environmental conditions; and management variables from the farmer interviews as predictors. To determine which treatment maximizes the provisioning of multiple ecosystem services at once (multifunctionality), we will use a multiple threshold approach to assess the number of services that reach different threshold levels for cover crop mixtures compared to monocultures. Finally, we will also use structural equation modeling (SEM) to estimate causal relationships between the set of farm management variables, N mass balance, environmental variables (e.g., soil texture, temperature, moisture), soil health measures, and the multiple ecosystem services that we measure. Objective 2: All functional traits have been analyzed except for leaf and root phosphorus concentrations, which we are preparing to complete at the beginning of the next project period. We are also currently analyzing field 2 particulate organic matter samples for carbon and nitrogen concentrations, which will complete our plot-level soil dataset. We plan to have comprehensive datasets for soil properties and plant traits spanning all fields included in this objective prepared for statistical analysis early in the next project period. To address Question 2.1 (which soil parameters best explain plant trait variation across farms spanning a gradient of soil health levels and N availability from SOM?), we will apply the same analytical approach as in Objective 1, using mixed-effects models to test for differences in plant traits (leaf N and P content, root:shoot, maximum height, and specific leaf area) by treatment and farm, and linear regression to identify soil health parameters that explain trait variation across the gradient. The plant trait information identified here will feed into the structural equation model to identify causal relationships between the multivariate dataset and ecosystem services, particularly legume N fixation. Objective 3: Approximately half of the farmer participants chose to complete the interview to collect the data needed to calculate costs and returns for planting cover crop mixtures within organic and transitioning management systems. We have created individual budgets for each of these farmers with a basic analysis of costs and returns, including marginal costs of cover cropping and cash crop yield and price for organic farmers. We are working on interpreting these results and will share them in the final project reports for farmers. Once we have the data on ecosystem services provided by different cover crop treatments, such as N supply from legume N fixation, or estimates of net C input to soil, we will expand the approach to include potential added returns from payments for ecosystem services schemes. This practice-based economic information will be shared in outreach activities with the farmer participant group and the wider farming community. We will prepare any extension outputs and recommendations for the Midwest Cover Crops Council cover crop decision tool in the final project year. ****Impacts**** What was accomplished under these goals? Our multi-institution research and extension project will use novel approaches to inform optimal cover crop mixture performance by: (i) conducting participatory research on farms to understand mixture performance along a soil health gradient; (ii) identifying relationships between plant functional trait diversity in cover crop mixtures and ecosystem services; and, (iii) generating management recommendations for organic farmers, including an economic assessment of cover crops, which will support broader use of cover crop mixtures in Michigan and beyond. Results will inform improvements to the Midwest Cover Crop Council's cover crop decision tool, and will be shared widely through outreach activities including extension bulletins, presentations at farmer meetings and conferences, field days, and engagement with policy communities. By identifying mixtures that improve ecological nutrient management on farms, results could be extended to address regional goals for improving water quality and reducing greenhouse gas emissions from agriculture. Objective 1: In an on-farm experiment, identify soil properties that predict ecosystem services and multifunctionality of cover crop mixtures. 1) Major activities Between April 27 - May 10, 2022 we sampled research plots from year 2 (field 2) for aboveground cover crop and weed biomass on 13 farms. The treatments (which we had planted between August 3-19, 2021), are: i) cereal rye; ii) crimson clover; iii) oat + cereal rye + crimson clover + dwarf essex rapeseed mixture; and iv) a "wildcard" (farmer-selected) mixture. Samples were analyzed for C and N concentrations, and a subset was prepared for stable isotope analysis to determine the amount of clover N derived from legume N₂ fixation. We received stable isotope results that allowed us to calculate crimson clover N fixation rates for year 1. We also received data on particulate organic matter (POM) N concentrations, which completed the year 1 soil dataset. We analyzed soil samples collected from field 2 for inorganic N, potentially mineralizable N, free and occluded POM fractions, potentially mineralizable C, permanganate oxidizable C, and soil texture. 2) Data collected Soils were analyzed for multiple soil health metrics as described in activities. We sampled aboveground cover crop biomass from field 2 in the spring. 3) Summary statistics and results Our analysis of soil data from both years and fields reveals large variation across farms and regions. In field 2, POM, which responds to farm management more quickly than total organic matter, varied 6-fold across all sites. Across both years, total soil C ranged from 0.6-4.4% and plant-available phosphorus ranged from 12-543 mg/kg, with similar means by region. These data will be used to determine relationships between soil health, cover crop traits, and ecosystem services. Spring 2022 biomass, field 2: In the Thumb, mean cover crop biomass was 1529 kg/ha for rye, 1718 kg/ha for crimson clover, and 1529 kg/ha for the 4-way mixture. In the mixture, crimson clover and cereal rye

produced similar biomass (~630 kg/ha each), with rapeseed producing less than half (272 kg/ha). In the southwest, mean biomass was 731 kg/ha for rye, 1366 kg/ha for clover, and 2538 kg/ha for the 4-way mixture. In the mix, clover produced the most biomass followed by rapeseed, with cereal rye producing much less (280 kg/ha). Year 1 vs Year 2: Considering fall and spring biomass across years, sole cereal rye performed better, on average, in year 2 than year 1 in the Thumb, but worse in the southwest. Sole clover produced less biomass in year 2 in both regions (year 1: 3220-3410 kg/ha; year 2: 1760-2380 kg/ha). In the southwest, 4-way mixture biomass and evenness was stable across years (mean: ~4500 kg/ha). In contrast, although the Thumb had a decrease in mean mixture biomass in year 2 (from 5500 to 4100 kg/ha), the mixture also shifted from being dominated by rapeseed in year 1 to strong oat growth in fall and a more balanced mixture in year 2. Clover N fixation and cover crop N recycling: In year 1 in the Thumb, clover acquired more aboveground N from N fixation in the mixture compared to monoculture (83% vs. 68%), while in the southwest there was no difference (~71% of plant N from fixation in both treatments). The total new (shoot) N supplied to fields from legume N fixation ranged from 20-95 kg N/ha in monoculture and from 3-57 kg N/ha in mixture. The mean N supply was ~60.5 kg/ha in sole clover and 25 in mixture. Total cover crop and weed biomass N content across treatments ranged from 9 (rye) to 121 (clover) kg N/ha, with a mean of 83 kg N/ha in the mixture, 95 kg N/ha in clover, and 39 kg N/ha in rye. 4) Key outcomes Our project has resulted in new applied knowledge of the ecosystem functions provided by cover crop mixtures on organic grain farms. Objective 2: Identify soil properties that predict variation in cover crop functional traits. 1) Major activities We sampled cover crop functional traits in rye, clover, and mixture treatments on 7 farms between April 27-May 9, 2022. Traits included maximum plant height, specific leaf area, leaf nutrient concentrations, and shoot C:N. We also measured root biomass to calculate root:shoot ratios, and analyzed roots for C and N concentration. 2) Data collected For field 2, we collected data on the cover crop traits listed in major activities in the spring. We collected belowground biomass samples and separated them into coarse, medium, and fine root classes based on root diameter. We also completed the soil analyses for year 2 fields, including soil inorganic N, mineralizable C and N, POM, and texture. 3) Summary statistics and results For year 2 soils, free and occluded POM ranged from 1.28-8.59 g/kg soil and 1.09-4.39 g/kg soil, respectively. There was a roughly 6- and 3-fold range in potentially mineralizable N (2.28-12.72 mg N/kg soil/week) and potentially mineralizable C (17.81-54.41 µg CO₂-C/g/day). Plant-available P also varied widely across sites, from 27-168 mg/kg soil. Across both study years and for all three species, root:shoot ratio was the most variable trait, followed by plant height. Dwarf essex showed the greatest variation in chemical traits, with a coefficient of variation (CV) of 44.9% for root %N, and 22.1% for leaf %N. Compared to dwarf essex, clover root %N and leaf %N varied less across sites (CV = 14.1 % and 12.1 %, respectively). Leaf %N for all three species was positively related to potentially mineralizable N ($p < 0.05$). There were also significant, negative relationships between soil clay content and clover root:shoot ($p = 0.0003$, $R^2 = 0.22$), rye fine root %N ($p = 0.002$, $R^2 = 0.17$), and dwarf essex specific leaf area ($p = 0.002$, $R^2 = 0.17$). 4) Key outcomes None to report Objective 3: Provide practical recommendations on how to best design and manage cover crop mixtures to maximize ecosystem services, including consideration of economic returns. 1) Major activities We interviewed 6 of the farmer participants in winter 2022. The interview guide collected farm economic data (i.e., partial budgets) to provide cost and return estimates for cover crop mixtures on organic farms. The budgets will predict the incremental net income the farmer would realize if they adopted one of the cover crop treatments used in the study at the field scale. The costs are being calculated relative to the typical cover crops each farmer plants (which varied). We are also calculating incremental net income relative to the cereal rye treatment to standardize results across farms. We also completed a review of the literature on payments for ecosystem services programs, which can be used to develop scenarios of added returns based on ecosystem services measured in the experiment. 2) Data collected Farm economic data to estimate changes to income with the cover crop treatments tested in this experiment. 3) Summary statistics None to report 4) Key outcomes None to report **Publications** Progress 09/01/20 to 08/31/21 Outputs Target Audience: We have continued to work with a group of organic and transitioning farmer partners for this research in two regions of Michigan, the "Thumb" and southwestern regions. All 14 farmers who participated in year 1 of the project were invited to continue with this on-farm research project for a second year with compensation provided in return for land use and their assistance with soil preparation for planting. In the second year, we worked with a group of 13 farmers. Two farmers in the southwestern region declined to continue, but we recruited a new participant in that region. In the second year of the study, farmers selected a new field of interest for the experiment. We planted the same three treatments from year 1, and farmers were invited to select a fourth treatment of either a 3- or 4-species mixture to test on their field; therefore, this fourth treatment differs across farms. Michigan's organic farmers are interested in opportunities to test various cover crop mixtures to enhance soil quality and diversify their crop rotations. Midwestern farmers who grow small grains have a unique window for planting cover crops following small grain harvest. This gap in crop production while the weather is still warm offers an opportunity to plant many cover crop types and mixtures. Changes/Problems: Despite the large logistical challenges of conducting research during the Covid-19 pandemic, we are pleased that we successfully planted the experiment on 14 farms in year 1, and 13 farms in year 2. However, aspects of all objectives were affected and/or delayed due to Covid-19. For instance, we exceeded transportation costs

included in our budget because researchers had to drive to field sites in individual vehicles to meet the safety requirements. We also had extra labor costs due to the additional time required for standard field and lab activities given extra safety requirements and restrictions on the number of people who can work in each space. We had to delay the in-person farmer interview components. However, we hope to complete most project activities in the next reporting period. What opportunities for training and professional development has the project provided? Training activities for this project period were focused on one-on-one work with mentors for the two PhD students and one master's student on the project. Both PhD students have a dissertation chapter that focuses on a sub-objective of this project, and in this period, they developed their dissertation proposals, and one of them passed her qualifying exams and defended her proposal. To complete field and lab work for the project we involved and trained a larger group of students: this included 10 Master's students from the PD's program, as well as 6 undergraduate students from the University of Michigan and 3 undergraduates and one graduate student from Michigan State University. Many of these students participated for a short period (e.g., several days during major field campaigns) in a volunteer capacity to gain research experience and training. Students gained experience in soil sampling and analysis techniques, as well as plant biomass and functional trait assessments. How have the results been disseminated to communities of interest? We hosted a virtual team meeting in February 2021 during which we provided a brief overview of the study and progress to date, including reporting initial results. The meeting was attended by farmer participants, the research teams from the University of Michigan and Michigan State University, and other agricultural professionals (extension agents and district conservationists in the regions where we are conducting the research). We then facilitated a discussion of perceptions of the experiment and cover crop performance to date, as well as shared goals and plans for the second year. In October 2021, we shared individual farm reports with all data from year 1 of the experiment, which included means and ranges for all measured soil health parameters and cover crop biomass results for each individual farm, along with ranges for their region. What do you plan to do during the next reporting period to accomplish the goals? Objective 1: We will complete the analysis of baseline soil samples from field 2 for a suite of soil health parameters this winter. From field 2, we will collect cover crop biomass data again immediately before incorporation in May, and we will send out tissue samples from both years for analysis of 15N to estimate N supply from clover N fixation. These data will be analyzed to address our two main research questions for this objective: Question 1.1. How do soil properties influence the performance of cover crop mixtures? And, Question 1.2. Which soil properties predict legume biomass and legume N fixation inputs in mixtures across farms? This winter, we will conduct an in-depth management interview with each farmer to quantify N and P mass balances and collect other management data that can be used as predictors in statistical models. We will test for treatment differences for individual ecosystem services using hierarchical linear mixed-effects models with treatment and year as fixed effects, and block nested in farm as a random effect. We will then use linear regression to identify variables that predict services (e.g., biomass, N fixation, N and P retention, weed suppression, etc.). The regression models will include soil health measures and other properties from soil analyses; environmental conditions; and management variables from the farmer interviews as predictors. To determine which treatment maximizes the provisioning of multiple ecosystem services at once (multifunctionality), we will use a multiple threshold approach to assess the number of services that reach different threshold levels for cover crop mixtures compared to monocultures. Finally, we will also use structural equation modeling (SEM) to estimate causal relationships between the set of farm management variables, N mass balance, environmental variables (e.g., soil texture, temperature, moisture), soil health measures, and the multiple ecosystem services that we measure. Objective 2: We are currently analyzing cover crop functional trait samples from year 1. Belowground biomass samples from year 1 will be analyzed for C, N and P concentrations. We are also in the process of analyzing soil samples collected at the individual plot level for field 2 from each farm. Analysis is currently in progress for extractable inorganic N, mineralizable C and N, particulate organic matter, POXC, and texture. In the spring, we will once again collect cover crop functional trait samples in treatments 1, 2, and 3 across the 8 farms included in year 2. We plan to have comprehensive datasets for soil properties and plant traits spanning all 14 fields included in this objective prepared for statistical analysis by the end of the next project period. To address Question 2.1 (which soil parameters best explain plant trait variation across farms spanning a gradient of soil health levels and N availability from SOM?), we will apply the same analytical approach as in Objective 1, using mixed-effects models to test for differences in plant traits (leaf N and P content, root:shoot, maximum height, and specific leaf area) by treatment and farm, and linear regression to identify soil health parameters that explain trait variation across the gradient. The plant trait information identified here will feed into the structural equation model to identify causal relationships between the multivariate dataset and ecosystem services, particularly legume N fixation. Objective 3: In the next project period, we plan to conduct interviews with all farmer participants to collect the data needed to calculate costs and returns for planting cover crop mixtures within organic and transitioning management systems. An environmental economist at UM, Dr. Michael Moore, will conduct the analysis for treatments in the on-farm experiment, aided by a graduate student at UM who is currently co-supervised by the PD. We will start the assessment with a basic analysis of costs and returns, including marginal costs of cover cropping and cash crop yield and price for both organic and transitioning farmers, following the farmer interviews.

Once we have the data on ecosystem services provided by different treatments, such as N supply from legume N fixation, or estimates of net C input to soil, we will expand the approach to include potential added returns from payments for ecosystem services schemes. To estimate these added returns, Moore and the graduate research assistant will use benchmark prices from existing programs or other incentives for conservation available in the Great Lakes region such as "avoided N" or C sequestration. This practice-based economic information will be shared in outreach activities with the farmer participant group and the wider farming community. In the spring or summer, we plan to hold a field day in each region to discuss research results, share farmers' perceptions of the experiment and cover crop management, and to identify priorities for future research projects.

Impacts
What was accomplished under these goals? Our multi-institution research and extension project will use novel approaches to inform optimal cover crop mixture performance by: (i) conducting participatory research on farms to understand mixture performance along a soil health gradient; (ii) identifying relationships between plant functional trait diversity in cover crop mixtures and ecosystem services; and, (iii) generating management recommendations for organic farmers, including an economic assessment of cover crops, which will support broader use of cover crop mixtures in Michigan and beyond. Results will inform improvements to the Midwest Cover Crop Council's cover crop decision tool, and will be shared widely through outreach activities including extension bulletins, presentations at farmer meetings and conferences, field days, and engagement with policy communities. By identifying mixtures that improve ecological nutrient management on farms, results could be extended to address regional goals for improving water quality and reducing greenhouse gas emissions from agriculture.

Objective 1: In an on-farm experiment, identify soil properties that predict ecosystem services and multifunctionality of cover crop mixtures. 1) Major activities Between April 14-May 13, 2021 we sampled research plots from year 1 (field 1) for aboveground cover crop and weed biomass. In year 2, we repeated the experiment in a second field (field 2) of approximately 0.3 hectares on 13 farms. The treatments, planted between August 3-19, 2021, are: i) cereal rye; ii) crimson clover; iii) oat + cereal rye + crimson clover + dwarf essex rapeseed mixture; and iv) a "wildcard" mixture, where each farmer selected from a list of 20 cover crops in three functional groups (legume, grass, or brassica). Treatments were planted with a grain drill in a randomized complete block design, with 4 replicate blocks per farm. Prior to tillage and planting, we collected a composite soil sample from each block per field in July 2021 to two depths: 0-20 and 20-40, and extracted subsamples for inorganic N and potentially mineralizable N (a biological indicator of soil health), and measured soil moisture and bulk density. In fall 2021, we analyzed subsamples for texture, permanganate oxidizable C (POXC, an indicator of soil biological health), total organic C and total N, macro- and micro-nutrients and pH. We also completed remaining analysis of soil health indicators for samples collected from field 1 (particulate organic matter and potentially mineralizable C). We sampled all field 2 plots (n=204) for cover crop and weed biomass in the fall before dormancy. 2) Data collected Soils were analyzed for multiple soil health metrics as described in activities. We sampled aboveground cover crop biomass from field 1 in the spring and from field 2 in the fall. 3) Summary statistics and results Our analysis reveals large variation across farms and regions in soil properties, which will be used to determine relationships between soil health, cover crop traits, and resulting ecosystem services. Particulate organic matter, which responds to farm management more quickly than total organic matter, varied 8-fold across all sites, while mean mineralizable C was 1.5 times higher on farms in the southwest than in the Thumb (42 v. 27 $\mu\text{g CO}_2\text{-C/g/day}$). In year 2, total organic matter ranged from 1 - 2.4% across farms, and plant-available phosphorus ranged from 12-146 mg/kg, with similar means by region. Spring 2021 biomass, field 1: In the Thumb, mean cover crop biomass was 1270 kg/ha for rye, 3009 kg/ha for crimson clover, and 3824 kg/ha for the 4-way mixture. In the mixture, on average, dwarf essex rapeseed produced the most biomass. In the southwest, mean biomass was 1786 kg/ha for rye, 2542 kg/ha for clover, and 2802 kg/ha for the 4-way mixture. In the mix, crimson clover and rapeseed produced similar biomass, with cereal rye producing much less (306 kg/ha). Fall 2021 biomass, field 2: In the Thumb, mean cover crop biomass at fall sampling was 1208 kg/ha for rye, 642 kg/ha for clover, and 2465 kg/ha for the 4-way mix. Oats dominated the mix in the fall (1600 kg/ha), with more biomass than rapeseed, rye, and clover combined. In the southwest, fall cover crop biomass was much lower than in year 1. Mean biomass for rye was 378 kg/ha, 391 kg/ha for clover, and 1822 kg/ha for the 4-way mixture. Rapeseed dominated the 4-way mix (897 kg/ha). 4) Key outcomes None to report.

Objective 2: Identify soil properties that predict variation in cover crop functional traits. 1) Major activities We sampled cover crop functional traits in treatments 1, 2, and 3 on 6 farms between April 26- May 14, 2021. Traits included maximum plant height, specific leaf area, leaf nutrient concentrations, and shoot C:N for all species. We measured root biomass to calculate root:shoot ratios. Following establishment of cover crops in field 2, we collected a second set of soil samples by individual treatment plot (n = 96) between September 10-17 on 8 farms. Shortly before the first frost (October 27-November 3), we measured plant heights in treatments 1, 2, and 3, and leaf N and shoot C:N ratio for oats in treatment 3, because this species winter-kills. 2) Data collected For field 1, we collected data on the cover crop traits listed in major activities by plot in the spring. We collected belowground biomass samples and separated them into coarse, medium, and fine root classes based on root diameter. We also completed the soil analyses for year 1 fields, including soil inorganic N, mineralizable C and N, particulate organic matter, POXC, and texture. For field 2, we measured soil moisture and total organic C and total N. In the fall before frost, we collected data

on functional traits, including maximum plant heights for all species in treatments 1, 2, and 3, and leaf N and C:N ratio for oats in treatment 3. 3) Summary statistics and results In year 1, there was a 6- and 12-fold range in POM N concentration across sites for free (13.7-89.5 mg/kg soil) and occluded (17.8 - 230.7 mg/kg soil) POM. Potentially mineralizable N and potentially mineralizable C also varied widely, with maximum and minimum values of 4.7 and 0.5 mg/kg soil, and 41 and 21 $\mu\text{g CO}_2\text{-C/g/day}$, respectively. Clover had the most variation in height across farms and treatments (11- 44.2 cm, mean=25.9 cm). Maximum height varied approximately 2-fold for both rye and dwarf essex. Mean specific leaf area (cm^2/g) was highest for clover (322.6) and lowest for dwarf essex (150.0) across plots. In field 2 (year 2), soil %N ranged from .08 - 0.16%, and soil %C ranged approximately 3-fold, from 0.71 - 2.3%. Soil P (Bray 1 P) ranged from 26 - 163 mg/kg soil. For oats in the 4-way mixture, maximum plant height ranged from 16 - 89 cm across farms. 4) Key outcomes None to report

Objective 3: Provide practical recommendations on how to best design and manage cover crop mixtures to maximize ecosystem services, including consideration of economic returns. 1) Major activities We completed the interview guide for this objective, which we will use to interview all farmer participants in winter 2022. Goals for the interview are to collect farm economic data (i.e., partial budgets) to provide cost and return estimates for use of cover crop mixtures compared to single-species cover crops on organic farms. The second goal is to use interview data combined with information gathered from the literature review on payments for ecosystem services programs to develop scenarios of added returns based on ecosystem services measured in the experiment. 2) Data collected Literature review to inform development of the questionnaire and budgeting approach, as well as information on payments for ecosystem services programs to inform scenarios to test. 3) Summary statistics None to report 4) Key outcomes None to report Publications Progress 09/01/19 to 08/31/20

PROGRESS

2019/09 TO 2020/08 Target Audience: We identified 14 organic and transitioning farmer partners for this research in two regions of Michigan, the "Thumb" and Southwestern regions, where there are relatively high numbers of field crop organic farmers. These farmers were invited to participate in this on-farm research project, noting their role, expectations, and compensation in return for land use and their work to prepare soil and assist with planting. Michigan's organic farmers seek information to enhance their farm management plans. They are also interested in opportunities to test various cover crop mixtures to enhance soil quality and diversify their crop rotations. Midwestern farmers who grow small grains have a unique window for planting cover crops following small grain harvest. This gap in crop production while the weather is still warm offers a quality time to plant and grow many cover crops types and combinations. Through a telephone questionnaire conducted by Morrone, the 14 participants were interviewed about their current knowledge and interest in cover crops and possible mixtures to test that would address multiple goals for their farm, including building soil health. Following the interviews, the research team identified three treatments to test on each farmer's field. Each farmer was also invited to select a fourth treatment of either a 3- or 4-species mixture to test on his field; therefore, this fourth treatment differs across farms. The four treatments are: i) cereal rye; ii) crimson clover; iii) the research team's mixture (oat + cereal rye + crimson clover + dwarf essex rapeseed); and iv) the farmers' "wildcard" mixture, where they selected from a list of 20 different cover crops from three functional groups (legume, grass, or brassica).

Changes/Problems: Despite the large logistical challenges of conducting research during the Covid-19 pandemic, we are pleased that we successfully planted the experiment on 14 farms in Michigan. However, aspects of all objectives have been affected and/or delayed due to Covid-19. For instance, we exceeded transportation costs included in our budget because researchers had to drive to field sites in individual vehicles to meet the safety requirements. We also had extra labor costs because it currently takes extra time to do standard field and lab activities due to extra safety requirements and restrictions on the number of people who can work in a given space. We have had to delay the in-person farmer interview components, and all in-person outreach activities are currently on hold. We hope to be able to hold these events in the second or third project year. We also had one methods challenge, which is common with on-farm research, where we were unable to use the same drill for planting cover crop treatments in both regions due to the cost of moving heavy equipment long distances. While both planters were seed drills, we were only able to control for seeding depth with one of them, which means there may be slight differences in planting depth across the two regions. However, we noted similar cover crop establishment in both regions at the fall sampling, and assume that this will not have a detectable effect on our results. We are attempting to resolve this issue for the second year of the study. What opportunities for training and professional development has the project provided? Training activities for this project period were focused on one-on-one work with mentors. The PDs trained three graduate students in soil sampling and analysis techniques, focused on learning how to measure different indicators of soil health relevant to N availability, and one graduate student in systematic literature review techniques. How have the results been disseminated to communities of interest? We shared baseline soil health information with all farmer participants by email. We will hold an online team meeting in February to share other initial project results and facilitate a discussion of perceptions of the experiment to date, and plans for the next phase. What do you plan to do during the next

reporting period to accomplish the goals? Objective 1: We are finishing our analysis of baseline soil samples for a suite of soil health parameters this winter. We will collect cover crop biomass data again immediately before incorporation in May, and send out tissue samples for analysis of ^{15}N . These data will be analyzed to address our two main research questions for this objective: Question 1.1. How do soil properties influence the performance of cover crop mixtures? And, Question 1.2. Which soil properties predict legume biomass and legume N fixation inputs in mixtures across farms? Farmer participants will select a second field on their farms in which we will repeat this experiment next year (2021-2022). This design allows for a two-year, on-farm study without altering farmers' rotation plans, since the primary window for a winter cover crop in Michigan follows small grain harvest. We will conduct in-depth management interviews with each farmer - once covid-related restrictions on in-person research are lifted - to quantify nutrient mass balances and collect other management data that can be used as predictors in statistical models. We will test for treatment differences for individual ecosystem services using hierarchical linear mixed-effects models with treatment as a fixed effect, and block nested in farm as a random effect. We will then use linear regression to identify variables that predict services (e.g., biomass, N fixation, N and P retention, weed suppression, etc.). The regression models will include soil health measures and other properties from soil analyses; environmental conditions; and management variables from the farmer interviews as predictors. To determine which treatment maximizes the provisioning of multiple ecosystem services at once (multifunctionality), we will use a multiple threshold approach to assess the number of services that reach different threshold levels for cover crop mixtures compared to monocultures. Finally, we will also use structural equation modeling (SEM) using the lavaan package in R to estimate causal relationships between the set of farm management variables, N mass balance, environmental variables (e.g., soil texture, temperature, moisture), soil health measures, and the multiple ecosystem services that we measure. Objective 2: To address Question 2.1 (which soil parameters best explain plant trait variation across farms spanning a gradient of soil health levels and N availability from SOM?), we will apply the same analytical approach as in Objective 1, using mixed-effects models to test for differences in plant traits (leaf N and P content, root:shoot, maximum height, and specific leaf area) by treatment and farm, and linear regression to identify soil health parameters that explain trait variation across the gradient. The plant trait information identified here will feed into the structural equation model to identify causal relationships between the multivariate dataset and ecosystem services, particularly legume N fixation. Objective 3: In the next project period we hope to be able to conduct interviews with all farmer participants to collect the data needed to calculate costs and returns for planting cover crop mixtures within organic and transitioning management systems. An environmental economist at UM, Dr. Michael Moore, will conduct the analysis for treatments in the on-farm experiment, aided by a graduate student at UM who is currently co-supervised by the PD. We will start the assessment with a basic analysis of costs and returns, including marginal costs of cover cropping and cash crop yield and price for both organic and transitioning farmers, following the farmer interviews. Once we have the data on ecosystem services provided by different treatments, such as N supply from legume N fixation, or estimates of net C input to soil, we will expand the approach to include potential added returns from payments for ecosystem services schemes. To estimate these added returns, Moore and the graduate research assistant will use benchmark prices from existing programs or other incentives for conservation available in the Great Lakes region such as "avoided N" or C sequestration. This practice-based economic information will be shared in outreach activities with the farmer participant group and the wider farming community. In February, we will hold a team meeting with the research team and farmer participants by Zoom to discuss research findings to date, perceptions of the experiment and cover crop management, and to refine plans for the next phase of the study. If Covid restrictions ease in summer 2021, we will plan two field days-- on one participating farm in each region of the study.

2020/09 TO 2021/08 Target Audience: We have continued to work with a group of organic and transitioning farmer partners for this research in two regions of Michigan, the "Thumb" and southwestern regions. All 14 farmers who participated in year 1 of the project were invited to continue with this on-farm research project for a second year with compensation provided in return for land use and their assistance with soil preparation for planting. In the second year, we worked with a group of 13 farmers. Two farmers in the southwestern region declined to continue, but we recruited a new participant in that region. In the second year of the study, farmers selected a new field of interest for the experiment. We planted the same three treatments from year 1, and farmers were invited to select a fourth treatment of either a 3- or 4-species mixture to test on their field; therefore, this fourth treatment differs across farms. Michigan's organic farmers are interested in opportunities to test various cover crop mixtures to enhance soil quality and diversify their crop rotations. Midwestern farmers who grow small grains have a unique window for planting cover crops following small grain harvest. This gap in crop production while the weather is still warm offers an opportunity to plant many cover crop types and mixtures. Changes/Problems: Despite the large logistical challenges of conducting research during the Covid-19 pandemic, we are pleased that we successfully planted the experiment on 14 farms in year 1, and 13 farms in year 2. However, aspects of all objectives were affected and/or delayed due to Covid-19. For instance, we exceeded transportation costs included in our budget because researchers had to drive to field sites in individual vehicles to

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To complete field and lab work for the project we involved and trained a larger group of students: this included 10 Master's students from the PD's program, as well as 6 undergraduate students from the University of Michigan and 3 undergraduates and one graduate student from Michigan State University. Many of these students participated for a short period (e.g., several days during major field campaigns) in a volunteer capacity to gain research experience and training. Students gained experience in soil sampling and analysis techniques, as well as plant biomass and functional trait assessments. How have the results been disseminated to communities of interest? We hosted a virtual team meeting in February 2021 during which we provided a brief overview of the study and progress to date, including reporting initial results. 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Objective 2: We are currently analyzing cover crop functional trait samples from year 1. Belowground biomass samples from year 1 will be analyzed for C, N and P concentrations. We are also in the process of analyzing soil samples collected at the individual plot level for field 2 from each farm. Analysis is currently in progress for extractable inorganic N, mineralizable C and N, particulate organic matter, POXC, and texture. In the spring, we will once again collect cover crop functional trait samples in treatments 1, 2, and 3 across the 8 farms included in year 2. We plan to have comprehensive datasets for soil properties and plant traits spanning all 14 fields included in this objective prepared for statistical analysis by the end of the next project period. 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fixation, or estimates of net C input to soil, we will expand the approach to include potential added returns from payments for ecosystem services schemes. To estimate these added returns, Moore and the graduate research assistant will use benchmark prices from existing programs or other incentives for conservation available in the Great Lakes region such as "avoided N" or C sequestration. This practice-based economic information will be shared in outreach activities with the farmer participant group and the wider farming community. In the spring or summer, we plan to hold a field day in each region to discuss research results, share farmers' perceptions of the experiment and cover crop management, and to identify priorities for future research projects.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? Our multi-institution research and extension project will use novel approaches to inform optimal cover crop mixture performance by: (i) conducting participatory research on farms to understand mixture performance along a soil health gradient; (ii) identifying relationships between plant functional trait diversity in cover crop mixtures and ecosystem services; and, (iii) generating practical management recommendations for organic and transitioning farmers, including an economic assessment of cover crops, which will support broader implementation of cover crop mixtures in Michigan and beyond. Results will inform improvements to a cover crop decision tool that is available by the Midwest Cover Crop Council, and will be shared widely through outreach activities including extension bulletins, presentations at farmer meetings and conferences, field days, and engagement with policy communities. By identifying mixtures that improve ecological nutrient management on farms, results could be extended to address regional water quality goals. Objective 1: In an on-farm experiment, identify soil properties that predict ecosystem services and multifunctionality of cover crop mixtures. 1) Major activities Fourteen farmers were recruited from two regions of Michigan with the assistance of organic agri-business representatives. On-farm research plots on these sites (7 in the southwestern region and 7 in the "Thumb" or eastern region) were established in a field where an overwintered grain was harvested in July 2020. We recorded GPS coordinates and field notes for all plots using an electronic data collection tool. Prior to grain harvest, we collected a composite soil sample in four replicate blocks per field in July 2020 to two depth increments: 0-20 and 20-40 cm. We immediately extracted soils for inorganic N pools and for potentially mineralizable nitrogen using an anaerobic incubation method (a biological indicator of soil health), and measured soil moisture and bulk density. In the fall, we analyzed the soil for texture, permanganate oxidizable C (POXC, an indicator of soil biological health), total organic C and total N, and for macro- and micro-nutrients and pH. Each experimental field size in the Thumb region is 0.36 ha. Farms in the Southwest region range from 0.06 ha to 0.33 ha (mean of 0.24 ha). The four treatments planted between August 4-21, 2020 are: i) cereal rye; ii) crimson clover; iii) the research team's mixture (oat + cereal rye + crimson clover + dwarf Essex rapeseed); and iv) the farmers' "wildcard" mixture, where they selected from a list of 20 different cover crops in three functional groups (legume, grass, or brassica). All treatments were planted with a grain drill in a randomized complete block design, with 4 replicate blocks per farm. We sampled all plots (n=224) for cover crop and weed biomass in the fall before dormancy. 2) Data collected Soils were analyzed for multiple soil health metrics as described in activities. We collected data on crop and weed cover at the time of soil sampling, and sampled aboveground cover crop biomass in the fall. 3) Summary statistics and results Our analysis reveals large variation across farms and regions in soil properties, which will be used to determine relationships between soil health, cover crop traits, and resulting ecosystem services. Total organic matter (%) to 20cm depth ranged from 1.1 to 5.6 % across farms, with higher levels in the Thumb, and significant differences among farms. Southwestern farms are sandier than farms in the Thumb. On average, southwestern farms contain 63% sand, while Thumb farms contain 56% sand. There are also large regional differences in pH (mean of 6.55 in the southwest versus 7.60 in the Thumb), but little variation between particular farms within each region. Plant-available phosphorus also varied greatly across farms with larger concentrations in the Southwest (151.2 mg/kg) compared to the Thumb (87.1 mg/kg), on average. Average POXC content at baseline soil sampling was higher across the Thumb farms (630 mg kg⁻¹soil) compared to southwest farms (380 mg kg⁻¹ soil). In the Thumb, cover crop biomass in the fall averaged 191 kg/ha for rye, 395 kg/ha for clover, and 1578 kg/ha for the 4-way mixture. In the 4-way mixture, on average, rapeseed produced the greatest biomass (1047 kg/ha), followed by oat (464 kg/ha), rye (98 kg/ha), and clover (76.2 kg/ha). In the southwest, biomass averaged 633 kg/ha for rye, 675 kg/ha for clover, and 1715 kg/ha for the 4-way mixture. Within the 4-way mixture, rapeseed produced the highest biomass (748 kg/ha), followed by oat (574 kg/ha), clover (202 kg/ha), and rye (191 kg/ha). 4) Key outcomes None to report. Objective 2: Identify soil properties that predict variation in cover crop functional traits. 1) Major activities We selected a subset of 7 farms for the functional trait objective, located in one region to minimize variation in climate and soil types. For those farms, we collected a second set of baseline soil samples by individual treatment plot (n = 84) between September 11-16, and are in the process of analyzing these samples for multiple soil health properties expected to predict variation in functional traits. We measured plant heights for each

species in treatments 1, 2 and 3, and leaf N and shoot C:N ratio for oats in treatment 3 because this species winter-kills. 2) Data collected We collected data on soil properties and plant functional traits by plot. We measured soil moisture and % total organic C and % total N, with additional analysis in progress for extractable inorganic N, mineralizable C and N, particulate organic matter, POXC, and texture. We collected data on plant functional traits in the fall before frost, including maximum plant height for all species in treatments 1, 2, and 3, and leaf N and C:N ratio for oats in treatment 3. 3) Summary statistics and results Across the seven trait farms, soil %N ranges approximately fourfold, from .09 % to 0.38 %. Similarly, soil % C shows considerable variation across farms, with a maximum value of 4.49 % and a minimum of 1.24 %. Soil P (Bray 1 P) ranges from 43.25 mg/kg soil to 107.25 mg/kg soil. Bulk density (g/cm³) ranges from 1.36 to 1.63 on these farms. For oats in the 4-way mixture, average height ranged from 17.2 cm to 40.3 cm across farms, with a CV of 0.282. There are significant differences in oat height across these farms ($p < 0.001$), which we expect to correspond with differences in soil parameters across farms. 4) Key outcomes None to report Objective 3: Provide practical recommendations on how to best design and manage cover crop mixtures to maximize ecosystem services, including consideration of economic returns. 1) Major activities Morrone interviewed farmers by phone to identify their knowledge and use of cover crops, and to discuss research plans. All farmers are certified organic, but several had land in transition so we were able to implement some trials on transitioning land. Of the fourteen farmers, only three do not use cover crops regularly. It was interesting to note that the farmers who did not use cover crops are dependent on manure and compost as sources of nitrogen. Typically, the farmers use cereal rye for winter cover as it is affordable, readily available and they are familiar with its establishment and performance. Some farmers had previously tried other covers including oilseed radish, oats, and Austrian winter pea. After the interview, farmers selected individual "wild card" treatments to test on their farms, which were combinations of 3 or 4 functionally-diverse cover crop types. We hired a graduate student research assistant to assist with the economic assessment; she began a literature review to develop the approach in fall 2020. 2) Data collected Farmer interview data on cover crop preferences and knowledge. 3) Summary statistics Nothing to report 4) Key outcomes Nothing to report **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 No publications reported this period.

2020/09 TO 2021/08 What was accomplished under these goals? Our multi-institution research and extension project will use novel approaches to inform optimal cover crop mixture performance by: (i) conducting participatory research on farms to understand mixture performance along a soil health gradient; (ii) identifying relationships between plant functional trait diversity in cover crop mixtures and ecosystem services; and, (iii) generating management recommendations for organic farmers, including an economic assessment of cover crops, which will support broader use of cover crop mixtures in Michigan and beyond. Results will inform improvements to the Midwest Cover Crop Council's cover crop decision tool, and will be shared widely through outreach activities including extension bulletins, presentations at farmer meetings and conferences, field days, and engagement with policy communities. By identifying mixtures that improve ecological nutrient management on farms, results could be extended to address regional goals for improving water quality and reducing greenhouse gas emissions from agriculture. Objective 1: In an on-farm experiment, identify soil properties that predict ecosystem services and multifunctionality of cover crop mixtures. 1) Major activities Between April 14-May 13, 2021 we sampled research plots from year 1 (field 1) for aboveground cover crop and weed biomass. In year 2, we repeated the experiment in a second field (field 2) of approximately 0.3 hectares on 13 farms. The treatments, planted between August 3-19, 2021, are: i) cereal rye; ii) crimson clover; iii) oat + cereal rye + crimson clover + dwarf essex rapeseed mixture; and iv) a "wildcard" mixture, where each farmer selected from a list of 20 cover crops in three functional groups (legume, grass, or brassica). Treatments were planted with a grain drill in a randomized complete block design, with 4 replicate blocks per farm. Prior to tillage and planting, we collected a composite soil sample from each block per field in July 2021 to two depths: 0-20 and 20-40, and extracted subsamples for inorganic N and potentially mineralizable N (a biological indicator of soil health), and measured soil moisture and bulk density. In fall 2021, we analyzed subsamples for texture, permanganate oxidizable C (POXC, an indicator of soil biological health), total organic C and total N, macro- and micro-nutrients and pH. We also completed remaining analysis of soil health indicators for samples collected from field 1 (particulate organic matter and potentially mineralizable C). We sampled all field 2 plots (n=204) for cover crop and weed biomass in the fall before dormancy. 2) Data collected Soils were analyzed for multiple soil health metrics as described in activities. We sampled aboveground cover crop biomass from field 1 in the spring and from field 2 in the fall. 3) Summary statistics and results Our analysis reveals large variation across farms and regions in soil properties, which will be used to determine relationships between soil health, cover crop traits, and resulting ecosystem services. Particulate organic matter, which responds to farm management more quickly than total organic matter, varied 8-fold across all sites, while mean mineralizable C was 1.5 times higher on farms in the southwest than in the Thumb (42 v. 27 $\mu\text{g CO}_2\text{-C/g/day}$). In year 2, total organic matter ranged from 1 - 2.4% across farms, and plant-available phosphorus ranged from 12-146 mg/kg, with similar means by region. Spring 2021 biomass, field 1: In the Thumb, mean cover crop biomass was 1270 kg/ha for rye, 3009 kg/ha for crimson

clover, and 3824 kg/ha for the 4-way mixture. In the mixture, on average, dwarf essex rapeseed produced the most biomass. In the southwest, mean biomass was 1786 kg/ha for rye, 2542 kg/ha for clover, and 2802 kg/ha for the 4-way mixture. In the mix, crimson clover and rapeseed produced similar biomass, with cereal rye producing much less (306 kg/ha). Fall 2021 biomass, field 2: In the Thumb, mean cover crop biomass at fall sampling was 1208 kg/ha for rye, 642 kg/ha for clover, and 2465 kg/ha for the 4-way mix. Oats dominated the mix in the fall (1600 kg/ha), with more biomass than rapeseed, rye, and clover combined. In the southwest, fall cover crop biomass was much lower than in year 1. Mean biomass for rye was 378 kg/ha, 391 kg/ha for clover, and 1822 kg/ha for the 4-way mixture. Rapeseed dominated the 4-way mix (897 kg/ha). 4) Key outcomes None to report. Objective 2: Identify soil properties that predict variation in cover crop functional traits. 1) Major activities We sampled cover crop functional traits in treatments 1, 2, and 3 on 6 farms between April 26- May 14, 2021. Traits included maximum plant height, specific leaf area, leaf nutrient concentrations, and shoot C:N for all species. We measured root biomass to calculate root:shoot ratios. Following establishment of cover crops in field 2, we collected a second set of soil samples by individual treatment plot (n = 96) between September 10-17 on 8 farms. Shortly before the first frost (October 27- November 3), we measured plant heights in treatments 1, 2, and 3, and leaf N and shoot C:N ratio for oats in treatment 3, because this species winter-kills. 2) Data collected For field 1, we collected data on the cover crop traits listed in major activities by plot in the spring. We collected belowground biomass samples and separated them into coarse, medium, and fine root classes based on root diameter. We also completed the soil analyses for year 1 fields, including soil inorganic N, mineralizable C and N, particulate organic matter, POXC, and texture. For field 2, we measured soil moisture and total organic C and total N. In the fall before frost, we collected data on functional traits, including maximum plant heights for all species in treatments 1, 2, and 3, and leaf N and C:N ratio for oats in treatment 3. 3) Summary statistics and results In year 1, there was a 6- and 12-fold range in POM N concentration across sites for free (13.7-89.5 mg/kg soil) and occluded (17.8 - 230.7 mg/kg soil) POM. Potentially mineralizable N and potentially mineralizable C also varied widely, with maximum and minimum values of 4.7 and 0.5 mg/kg soil, and 41 and 21 $\mu\text{g CO}_2\text{-C/g/day}$, respectively. Clover had the most variation in height across farms and treatments (11- 44.2 cm, mean=25.9 cm). Maximum height varied approximately 2-fold for both rye and dwarf essex. Mean specific leaf area (cm^2/g) was highest for clover (322.6) and lowest for dwarf essex (150.0) across plots. In field 2 (year 2), soil %N ranged from .08 - 0.16%, and soil %C ranged approximately 3-fold, from 0.71 - 2.3%. Soil P (Bray 1 P) ranged from 26 - 163 mg/kg soil. For oats in the 4-way mixture, maximum plant height ranged from 16 - 89 cm across farms. 4) Key outcomes None to report Objective 3: Provide practical recommendations on how to best design and manage cover crop mixtures to maximize ecosystem services, including consideration of economic returns. 1) Major activities We completed the interview guide for this objective, which we will use to interview all farmer participants in winter 2022. Goals for the interview are to collect farm economic data (i.e., partial budgets) to provide cost and return estimates for use of cover crop mixtures compared to single-species cover crops on organic farms. The second goal is to use interview data combined with information gathered from the literature review on payments for ecosystem services programs to develop scenarios of added returns based on ecosystem services measured in the experiment. 2) Data collected Literature review to inform development of the questionnaire and budgeting approach, as well as information on payments for ecosystem services programs to inform scenarios to test. 3) Summary statistics None to report 4) Key outcomes None to report **PUBLICATIONS (not previously reported):** 2020/09 TO 2021/08 No publications reported this period. ** **

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Management Techniques to Optimize Soil Ph and Nutrient Availability in Organic Highbush Blueberry Grown East of the Cascade Range

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NON-TECHNICAL SUMMARY

In the Pacific Northwest, Oregon and Washington produced 228 million pounds of blueberries in 2017, totaling 38% of the national crop. Organic blueberry production is predominately located in the semi-arid region east of the Cascade Range. While the size of the industry has increased, research support has not. Consequently, many of the horticultural issues that have emerged remain unresolved, specifically regarding nutrient management. Growers report that managing soil pH and organic matter are their foremost concerns that limit production and long-term viability. Blueberry requires very specific pH and organic matter conditions. There are no research-based recommendations to advise growers on how to best modify soil pH and provide organic matter. The goal of this project is to develop optimized nutrient strategies to facilitate industry expansion and environmental stewardship. Specifically, we are proposing to evaluate modified practices including (obj. 1) the novel use of pelleted sulfur as a slow-release method, (obj. 2) acidified grape pomace, and (obj. 3) alternative biochar feedstocks produced at reduced temperatures for lower pH. These objectives are intended to relieve industry bottlenecks to improve pH and organic matter in organic blueberry. Research will be complimented with (obj. 4) a complete cost-benefit analysis to determine the economic impacts and viability of investigated practices. We will develop (obj. 5) rigorous educational programs related to planting establishment and nutrient management, including dissemination of project information and evaluation of impact. This project will provide producers in this region and throughout the U.S. with valuable applied outcomes to optimize production.

OBJECTIVES

Objectives: The long-term goal of this project is to develop improved horticultural management strategies for soil pH and organic matter within the unique conditions in eastern Oregon and Washington. This will ensure the short- and long-term success of the robust organic blueberry industry in eastern Washington and Oregon. This goal is essential to supporting the primary organic blueberry production region in the US, as well as creating more opportunity for regional expansion. Outcomes from the project also will be applicable to other production regions which face similar challenges. Project priorities have been developed collaboratively with stakeholders and will focus on developing methods to economically modify and maintain soil pH and organic matter, both of which are industry bottlenecks imperative for existing and future production success. Currently, there is no research-generated information to guide organic growers with naturally high pH soils on best practices to manage soil pH

and organic matter. Based on our previous work and preliminary studies, we are proposing to evaluate modified horticultural practices, such as the use of pelleted and micronized sulfur for rapid and long-term soil acidification and the use of acidified grape pomace and alternative biochar feedstocks as improved sources of organic matter within these naturally alkaline soils. To accomplish the goal of developing novel and improved approaches to modify soil pH and organic matter in organic blueberry production, we will complete the following research and outreach objectives: Provide a long-term strategy for quickly reducing and maintaining soil pH for organic blueberry through the evaluation of new formulations and methods of sulfur application techniques. Determine if composted grape pomace, alone or with an acidifying agent, and surface-applied or incorporated, is a suitable organic matter amendment for organic blueberry. Determine if biochar produced from prunings is suitable and cost-effective for increasing organic matter and subsequent nutrient management by raising carbon content in organic blueberry fields. Complete a cost-benefit analysis to determine the economic impacts and viability of investigated practices. Develop region-specific educational programs related to planting establishment and nutrient management, including the dissemination of project information and evaluation of impact.

APPROACH

Objective 1. Provide a long-term strategy for quickly reducing and maintaining soil pH for organic blueberry through the evaluation of new formulations and methods of sulfur application techniques. A 2 x 6 factorial experiment will be arranged at HAREC in a split-plot design with two post-plant water acidification treatments as main plots, and six sulfur applications as subplots, replicated four times. Individual treatment plots will consist of eight blueberry plants. Each main plot will be randomly divided into two plots that will be irrigated after planting with either acidified or non-acidified water produced from a sulfur burner system. Subplot S₀ pre-plant treatments in year one will consist of: 1) growers standard soil incorporated "wet" prilled S₀ (control); 2) drip injected micronized wettable S₀; 3) soil incorporated dry prilled S₀; 4) drip injected micronized S₀ with soil incorporated dry prilled S₀; in addition to the year one treatments, we will evaluate the multiyear combinations of, 5) soil incorporated "wet" prilled S₀ (yr. 1) with post-plant surface prilled S₀ (yr. 2&3) and; 6) drip injected micronized S₀ (year 1) with post-plant surface prilled S₀ (yr. 2 and 3) (Table 2). Both wet and dry incorporated prilled S₀ (Tiger 90CR, 0N-0P-0K-90S; Tiger-Sul products LLC, Sheldon, CT) applications will follow a rate of application based off of the soil test results from the experimental field at HAREC (site pH = 7.2). Wettable micronized S₀ (0N-0P-0K-80S; Nufarm Americas Inc., Burr Ridge, IL) will be evaluated at 150 kg ha⁻¹ determined by Almutairi et al. (2017b) in the evaluation of micronized S₀ in western Oregon blueberry production systems. In years two and three, treatment rates of surface applied prilled S₀ will be based on soil test results. **Objective 2.** Determine if composted grape pomace, alone or with an acidifying agent, and surface-applied or incorporated, is a suitable organic matter amendment for organic blueberry. Screenhouse trials. Three un-amended field soils will be collected from Washington (Quincy sands and Warden silt loams) and Oregon (Atkins sandy loam) during Sept. 2019. These soil types are representative of what growers east of the Cascade Range utilize for blueberry production. Soils will be divided into 4 treatments per soil type and tested for organic matter content, initial pH, and free lime content before treatment application. Treatments include: 1) unamended soil; 2) soil + CGP; 3) soil + CGP + elemental sulfur in micronized form; and 4) soil + CGP + prilled sulfur pellets (TIGER 90CR® Organic Sulfur). Field trial, Based on the initial results of the screenhouse experiment, we will select the most promising treatment and integrate it into a field trial with a grower cooperater in spring 2021. Compost will again be sourced from our local supplier in Grandview, WA and chemically analyzed for total carbon and nitrogen, pH, EC, soluble ions, and the compost's acidification requirement (Costello and Sullivan, 2014). We will select a site planting blueberry that spring and has already undergone pre-plant soil acidification using standard grower practices, thus focusing the scope of this experiment to assess whether CGP is a suitable organic matter source for organic blueberry. The experimental design will be a randomized complete block with four replications of three treatments. **Objective 3.** Determine if biochar produced from prunings is suitable and cost-effective for increasing organic matter and subsequent nutrient management by raising carbon content in organic blueberry fields. Greenhouse trial A series of controlled experiments will be conducted in a greenhouse at HAREC to evaluate potential feedstocks for production of biochar that are suitable for blueberry. To do so, we will utilize prunings from blueberry fields, as well as from apple orchards and vineyards, which are plentiful in the region, to produce the biochars. Each biochar will be created at a low pyrolysis temperature (300-350 °C) using an industrial pyrolysis unit (Biological Carbon LLC). Lower pyrolysis temperature (\leq 350 °C) reduces ash content and results in less alkaline biochars (Ippolito et al., 2014; Zhao et al., 2017). The physiochemical properties of the biochars, including surface area, pH, EC, and content of ash, C, N, and other nutrients, will be characterized using protocols established by the International Biochar Initiative (IBI, 2015). The biochars will be mixed at 10% and 20%, by volume, with Quincy sands and Warden silt loam soils, and added to 4-L pots and planted with 'Duke' blueberry. Field trial The biochar deemed most conducive to growth in the greenhouse will be evaluated more rigorously under field conditions. The site will be located in a certified organic field at HAREC and acidified prior to planting with prilled and/or micronized S⁰. Treatments will be laid out in a randomized complete block design

with five replicates and include rows of 'Duke' blueberry grown with wood chips (control) or biochar applied by one of two methods. Objective 4. Complete a cost-benefit analysis to determine the economic impacts and viability of investigated practices. For each trial, all expenses will be categorized, application rates of production inputs will be monitored and recorded, and crop yield in year 3 will be collected for each treatment combination by Galinato and PDs Lukas and DeVetter and co-PD Bryla. From these data, the value of production for each treatment combination will be estimated. These figures will also be compared to the enterprise budget generated by Galinato et al. (2016), which is the most current guide on the costs for establishing and producing organic blueberries in eastern Washington and serves as the baseline for the economic analysis. The results will be used to assess the economic viability of the investigated treatments. Objective 5. Develop region-specific educational programs related to planting establishment and nutrient management, including the dissemination of project information and evaluation of impact. We have developed a dynamic outreach plan to communicate project results to stakeholders and the larger public. We will hold and participate in annual field days starting in 2020 in Oregon and Washington, whereby we will introduce the project and provide project updates. The field days will be held at the HAREC, which will allow stakeholders to observe and discuss the treatments with the research team. In addition, we will present project results at annual blueberry field days hosted by Strik at the North Willamette Research and Extension Center in Aurora (western Oregon). In Washington, we will also present results at the annual field day held at the Northwestern Washington Research and Extension Center in Mount Vernon and hold field days in 2021 and 2022 in Prosser, Washington, where eastern Washington growers are concentrated. This strategy will ensure that there are multiple opportunities for both Oregon and Washington growers across the state to attend field days and learn about the project. We will also report project results at the WA Small Fruit Conference in 2021 and 2022, the Oregon Blueberry Conference in 2020 and 2022, the Hermiston Farm Fair in 2022, OTCO Organicology in 2021 and the Organic Farming research conference at Ecofarm. Final project results will be shared at the 2022 American Society for Horticultural Science Conference, which will educate a broad national audience of horticultural scientists and extension specialists. Progress 09/01/19 to 08/28/23

Outputs Target Audience: University and government researchers, university extension specialists, private agricultural consultants, horticultural crop growers and other industry stakeholder, and graduate and undergraduate students. Changes/Problems: No changes or problems to report in 2023 What opportunities for training and professional development has the project provided? This project provided training opportunities for two graduate students at Washington State University (Qianwen Lu and Brenda Madrid, both now graduated) and technical support (Sean Watkinson, Emma Rogers, and Brian Maupin) on executing a long-term greenhouse study. At Oregon State University and the USDA, three graduate students were trained (Andrea Retano, Alexander Gregory, and Pooja Kumari (two graduated and one expected Fall 2023) on this project. Students conducted greenhouse, field experiments on compost, biochar, and soil acidification as well as engaged in extension efforts. How have the results been disseminated to communities of interest? Information has been shared with growers and industry stakeholders, university and government researchers, university extension specialists, private agricultural consultants, and graduate and undergraduate students at academic conferences, grower meetings, field visits, and one-on-one. As proposed, we have shared project results through an eOrganic webinar, the Hermiston Farm Fair, the American Society for Horticultural Science, the International Plant Propagators Society, the Soil Science Society of America annual meetings in 2021 and 2022, and the Washington Blueberry Commission meeting in 2022, and other regional media outlets. These were attended by growers, students, researchers, and other stakeholders. Many results were disseminated to blueberry growers in eastern Washington primarily through one-on-one conversations due to this project occurring during the pandemic and the small concentration of growers in the area (three growers represent nearly all of the organic blueberry production in eastern Washington). What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported Impacts What was accomplished under these goals? This objective was evaluated in a new 0.24-hectare (ha) planting of northern highbush blueberry (NHB) 'Duke', between Spring 2020 and Fall 2022 at Oregon State University's Hermiston Agricultural Research and Extension Center (HAREC). The S treatments were with or without supplemental acidification using an organic-approved S burner. Soil S application treatments included: 1) grower standard of applying S to the soil surface eight months prior to planting; 2) fertigation via the drip irrigation system of micronized S over eight weekly applications after planting; 3) soil incorporation of S prills directly before planting without the allowance of the standard fallow period; 4) soil incorporation of dry S prills directly before planting followed by 8 weeks of fertigation with micronized S; 5) incorporation of S prills as in the control followed by surface application of S prills in spring of year two; 6) and micronized S application directly after planting followed by annual surface application of S prills in spring of year two. Soil pH was lower in plots receiving acidified irrigation water than nonacidified water from fall 2020 (5.66 vs. 6.09), until fall 2022 (5.43 vs. 6.05), compared to native soils at 7.2. Average yield was greater in plots receiving acidified water compared to the receiving unacidified water over the 2021 and 2022 seasons, producing 21.4% and 15.9% greater berry yields, respectively. These results suggest acidification of irrigation water remains a beneficial soil pH management practice for NHB plantings in calcareous soils. The grower standard of allowing a fallow period prior soil incorporation of S does not meaningfully improve soil pH management or overall berry

yields during establishment. These findings support a decreased establishment costs, as fields can be brought into production more quickly relative to current standard practices. This objective was completed through a series of greenhouse experiments that evaluated composted grape pomace (CGP), CGP + prilled sulfur pellets, CGP + micronized sulfur, leonardite, and a zero-amendment control in three soils collected from Washington and Oregon using tissue culture NHB 'Aurora' plants. Overall, CGP showed no deleterious effects on blueberry growth compared to the control and may be a suitable pre-plant amendment in blueberry field sites low in soil potassium (K) and post plant as a mulch if leaf tissue levels are at or below sufficiency levels for K. CGP electrical conductivity and pH should also be closely monitored to avoid deleterious effects on blueberry growth and productivity. Micronized sulfur reduced pH more rapidly than prilled sulfur and would be a suitable acidifying agent for CGP. Information has been shared with growers interested in using CGP through one-on-one conversations and an extension newsletter article disseminating findings is planned for winter 2023. A 0.2-ha field trial was established at OSU HAREC as planned to determine the effects co-composting and placement of biochar on plant growth and early fruit production in new organic planting of highbush blueberry. Biochar was used "as is" or co-composted with grape pomace and either applied in a band or as a thin layer on the soil surface of the rows prior to shaping the planting beds. Additional treatments included apple wood chips (industry standard) and compost only, which were likewise incorporated into the planting beds. Our results showed that compost incorporated treatment showed best soil nutrient availability, soil carbon, and other soil biological characteristics. In addition, in two years, incorporated treatments showed better nutrient availability than treatments applied in bands for all the amendments. Biochar treatments did not show better results than compost treatments in one year, but it did show better results than the control (woodchips). We have presented the data from this study at the annual Soil Science Society of America conference, in an eOrganic webinar, at the Hermiston Farm Fair, and at the annual Washington Blueberry Commission meeting. Using data from the field studies, estimates of S application costs (labor and material) and irrigation labor costs were generally lower by 37% to 72% when irrigating without acidified water. These costs were lower mainly due to irrigation labor requirements, which were one-sixth that of the alternative (i.e., with acidified water). We used partial budget analysis to estimate the impacts of different practices on farm income (during Year 3 of production). The baseline of the economic analysis was the grower standard of "soil incorporated wet prilled S using irrigation with acidified water." Field studies reported increases in crop yield relative to the baseline for three alternative practices: soil incorporated wet prilled S; drip injected micronized S with soil incorporated dry prilled S; and soil incorporated wet prilled S in Year 1 with post-plant surface prilled S in Years 2 & 3. All three used irrigation without acidified water. The net change in gross margin is positive in these alternatives, implying that they are, potentially, more profitable than the current practice. Their estimated increments to the baseline gross margin were \$1,556, \$1,474, and \$1,263 per acre, respectively. Gross margin is the return above variable costs. At minimum, the producer must be able to cover their total variable costs to continue production. The results highlight the economically feasible potential of managing soil PH in organic blueberries without the need for acidified water and considering the three aforementioned formulations and methods of sulfur application techniques. Using data from the biochar field studies, estimates of the application costs (material and labor) of all alternative practices were higher than the baseline (i.e., wood chips) by 22% when using compost, 37% when using biochar and compost, and 94% when using biochar. The differences in the costs were due to the material prices, where biochar was the most expensive. In terms of measuring the impacts of the investigated practices on farm income (during Year 3 of production), the field studies reported a crop yield increase of 9% (biochar band) to 45% (compost incorporated) relative to baseline yield, except the "compost band" practice where it was 13% lower than the baseline yield. The yield increase translated to increases in gross return and gross margin. Partial budget results showed that all alternative practices, except compost band, have potential to be more profitable than using wood chips. Their estimated increments to the baseline gross margin per acre were \$738 in biochar band, \$2,214 in biochar + compost incorporated, \$2,788 in biochar + compost band, \$984 in biochar incorporated, and \$3,689 in compost incorporated. The results underscored the potential of biochar as a more economically feasible soil amendment than the typical practice. In interviews with producers, soil pH and nutrient management were identified as key with most using S burners to lower pH post-plant. Updating nutrient management and fertilizer recommendations to reflect the region was also identified as a need. The project has disseminated numerous publications that address the needs identified by key producers, including newsletters on dried S prills lowering soil pH, newsletters on experimental approaches to nutrient management that can decrease application timelines, prominent grower publications, Extension publications, and numerous presentations. Presentations have yielded follow up contact with producers for further information to help disseminate and educate on project results that address their identified concerns. Additionally, project participants have engaged with growers extensively to disseminate results that address key concerns identified during evaluation. Publications Progress 09/01/21 to 08/31/22 Outputs Target Audience: Horticultural growers, agricultural consultants, and university and government researchers. Changes/Problems: Field-based activities in objective 2 were re-structured due to unwanted treatment interactions with plant nutrients. Specifically, the grape pomace elevated soil and blueberry leaf potassium levels beyond sufficiency ranges and the planting was at risk

of yield declines due to elevated potassium. We likewise observed elevated pH and soil K levels (ranges measured on 25 Oct. 2021: pH 6.3-7.9; soil K 308-1098 mg/kg) in our media and did not want to apply these treatments in a commercial field and risk crop damage. As such, we developed a field trial with different organic matter particle size distributions to re-direct the objective to complete our goals for the project without risk of compromising grower field productivity. What opportunities for training and professional development has the project provided? The project has provided professional development opportunities for undergraduate and graduate students, and post-doctoral and technical staff. Students and staff have participated in professional conferences where they were able to learn new skills, network with other researchers, and share their work. Additionally, regional project meetings have created opportunities for our team to engage with other research, extension, and industry personnel to develop practical skills. How have the results been disseminated to communities of interest? Stakeholders have been engaged in the project through regional outreach activities where project results have been shared. One-on-one interactions have proven to be the most beneficial and common in 2022. Growers and industry professionals have visited research sites, and are engaged in the project outcomes. Additionally, quarterly newsletters are shared with large stakeholder networks to provide project updates and preliminary outcomes. Lastly, growers were engaged through focus group sessions (OBJ 5) to help identify bridges and barriers to organic blueberry production and to help the project team tailor information dissemination to best support the active producer population. What do you plan to do during the next reporting period to accomplish the goals? In the next and final reporting period, all objectives proposed in the project will be completed. In that time, final data collection will occur and data will be analyzed to produce extension and peer review publications. The project team will also strategize on the best routes to further the significant field trials to continue to collect long-term data beyond the scope of this project. Impacts What was accomplished under these goals? OBJ 1. All proposed methods for this objective for years 1-3 have been completed. The OSU HAREC field site was amended with grower standard practices in November 2019, with experimental treatments initiated in March 2020. Main plot treatments consisted of two post-plant water acidification treatments, randomly divided into two plots that are irrigated with either acidified or non-acidified water produced from the sulfur burner system three times per week. Sub plots consisted of six sulfur applications as subplots with 10 plants/ subplot. Soil samples were collected for analysis of nutrient status in the Spring and Fall of year 3 with laboratory processing of the Fall sample lot currently being performed. Plant growth was assessed by visual rating of relative plant vigor and plant height, which was assessed with 3D-models using UAS-based RGB imagery to estimate plant height and volume. Spectral character of blueberry plant leaves was assessed on a monthly basis, and compared against leaf spectral estimations collected manually using a SPAD greenness meter, and leaf tissue nutrient content. Fruit parameters including fruit quality (titratable acidity, firmness, mass per 100 berries, diameter, and brix) and total yield were assessed for year 3. Preliminary data for year 3 indicate similar trends as those identified in year 2. In terms of soil pH, plots receiving regular applications of acidified water by means of the elemental sulfur burner have shown, on average, a greater degree of soil acidification than plots receiving exclusively unacidified irrigation water. Application of dry Sprills directly before planting appears to maintain soil pH within the desired range of 4.5 to 5.5 for a longer duration than the grower standard of applying Sprills 8 months prior to planting. Treatments which received micronized Sin year 1 as their only soil pH treatment continue to exhibit soil pH problems, which have not been fully rectified by application of acidified irrigation water or surface application of Sprills in year 2, though surface application of Sat a rate of 500lbs/acre does appear to result in a decrease in soil pH over the following 12-month period. Surface application of Swas not performed in year 3 to avoid potential over-acidification of treatment plots. Plant vigor, height, volume, and nutrient status follow trends which closely match those of soil pH, with plants experiencing high pH being on average smaller, less vigorous, and displaying leaf nutrient status indicative of pH problems such as low iron concentration. These observations, which will be fully quantified and analyzed by Fall 2022. OBJ 2. A 2021 greenhouse trial with composted grape pomace was established at WSU NWREC and remains underway in 2022. Initial soil samples as well as samples collected at 2, 4, and 8 weeks and 6 and 9 months were sent to a commercial laboratory. Samples were analyzed for pH, free lime content, carbonates, organic matter, total C and N, EC, soluble ions, and macro- and micro-nutrients. Monthly leachate data collection and measurement of leachate pH and EC have also been ongoing. After approximately one year and allowing soil pH to modify, tissue-culture transplants of 'Aurora' were added to bioassay containers on 4 Feb. 2022. Plant height was recorded weekly for 4 weeks. Destructive sampling where biomass of roots and shoots, as well as soil and plant nutrient analysis, will be done in Oct. 2022. Our original plan was to select the most promising treatments from the greenhouse trial using composted grape pomace and scale up into field trials in eastern Washington in 2022. Unfortunately, our previously-identified grower collaborator was not able to host this trial and cited previous experience with composted grape pomace as being concerning. Discussions with this grower led us to establish a different, yet similar and relevant trial to conduct throughout the remainder of the project period described below. Organic blueberry growers east of the Cascade Range use orchard wood chips as their primary source of organic matter. Growers west of the Cascade Range use sawdust from Douglas fir, which has a small particle size relative to the orchard wood chips. The question we are addressing in this second experiment is the role of particle size on soil

organic matter and plant growth using amendments available to organic blueberry growers east of the Cascade Range. Unamended soil representative of commercial blueberry production was collected 2 May 2022 in Prosser, WA and tested for pH, OM, EC, N, P, K, Ca, and C. Aged orchard wood chips were also obtained from a commercial grower on 23 March 2022. Treatments were applied in a randomized complete block with five replications. The three treatments included: 1) 50% soil 50% full-sized orchard wood chips; 2) 50% soil and 50% fine wood chips; and 3) Soil control (no wood chips). Tissue-culture 'Aurora' plants not used for our grape compost experiment were planted (planting May 2022). Height, biomass, and nutrient data will be collected similar to the first greenhouse trial with destructive sampling scheduled for Oct. 2022. OBJ 3. A 0.4-acre field trial was established in 2021 at OSU HAREC as planned to determine the effects co-composting and placement of biochar on plant growth and early fruit production in new organic planting of highbush blueberry 'draper'. Biochar was used 'as is' or co-composted with grape pomace and either applied in a band or as a thin layer on the soil surface of the rows prior to shaping the planting beds. Starting 2022, we collected plant height data and no differences among treatments were seen. The plants were pruned for fruit production in 2022. Prior to pruning, bud set data was collected and no differences among treatments were found. On an average, the bud set for all the treatments was 23%. DNA extraction for bacterial and fungal community structure for 2021 and polymerase chain reactions are completed, and samples are ready to be sequenced. Another set of soil samples were collected in May 2022 and analyzed for pH, free lime content, carbonates, organic matter, total C and N, EC, soluble ions, and macro- and micro-nutrients. We plan to collect infiltration rate measurements, water holding capacity, soil microbial assessments, soil carbon fractions, nutrients, and microbial community structure for soil samples that will be collected in Fall 2022. We have harvested berries from the plants this year and are collecting data on titratable acidity, Brix, nutrient, berry weight and diameter and overall yield. Plant growth is also being assessed by visual ratings of vigor and height, as well as leaf tissue nutrient content, leaf chlorophyll levels, and multispectral aerial imagery. OBJ 4. Preliminary data have been collected for the cost-benefit analysis of the practices outlined in Obj 1-3, particularly application rates of production inputs and crop yield for each treatment. The comparison of net economic benefits of alternative practices relative to the standard (baseline) practice will be drafted during Fall 2022, when additional data are received after harvest in summer 2022. OBJ 5. Assessments of current practices with Washington and Oregon growers were planned to occur in August and October 2022 and led by co-PI Rebecca Sero. A list of interview questions was developed with PI Lukas and co-PIs DeVetter and Hoheisel. The questions were submitted to the WSU Internal Review Board (IRB) to receive exemption from full IRB review status. Sero left WSU and Season Hoard (Project Manager at WSU and recommended by Sero) replaced her. Five semi-structured interviews with organic and conventional blueberry producers in eastern Washington and western Oregon were conducted via Zoom videoconference in February of 2022. Information from these interviews will be leveraged for later ripple mapping in 2023, before project termination. Publications Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Singh, S., Retano, A., Lukas, S., Bryla, D. Responses of soil microbial community structure and activity to various organic amendments in blueberry systems. American Society of Agronomy-Crop Science Society of America-Soil Science Society of America annual meetings, Baltimore, MD (November 6-9, 2022). Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Gregory, A., Lukas, S., Orr, S., Bryla, D. 2021. Response of six cultivars of highbush blueberry to different rates of humic acids in hydroponic solution. HortScience 56 (9) pp S189. Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Kumari, P., Lukas, S., Singh, S., Bryla, D. 2021. Assessment of feedstock materials for producing a suitable and cost-effective biochar to use as a soil amendment for northern highbush blueberry. HortScience 56 (9) pp S194. **Progress** 09/01/20 to 08/31/21 **Outputs** Target Audience:Horticultural growers, agricultural consultants, and university and government researchers. Changes/Problems:Co-PI DeVetter's experiment is still delayed by 1 year due to two factors: 1) having to make the grape pomace compost rather in 2019/2020 than purchasing finished compost because none was locally available that met the conditions required by the experiment and 2) the onset of the pandemic in 2020 halted the start of all new experiments at WSU and she could not resume operations on this study until July 2020. What opportunities for training and professional development has the project provided?Professional training for project personnel, for this reporting year, has included field research and extension opportunities for three graduate students and one post-doctoral researcher. These students have all been able to directly engage in data collection and experimental design for greenhouse and field research, as well as present data to professional colleagues and regional stakeholders. How have the results been disseminated to communities of interest?Preliminary results and project findings have been shared with grower stakeholders at experimental sitefield visitsand field days within Oregon. Results have also been shared at National conferences. What do you plan to do during the next reporting period to accomplish the goals?During the next year of the project, we intend to continue laboratory and field research activities. In the next year, we anticipate results will be in a stage that will be ready for academic manuscript drafting. Aside from academic publications results will be disseminated on project websites, eOrganic, and at conference events. In 2022 we plan to graduate one of the MS students working directly on this project. **Impacts** What was accomplished under these goals? OBJ 1. All proposed

methods for this objective for years one and two have been completed. The OSU HAREC field site was amended with grower standard practices in November 2019, all control and experimental treatments were initiated in March 2020. Irrigation, injectors, and the water acidification sulfur burner were installed in March. "Duke" blueberry plants were planted in the field on 2 Apr. 2020 in a 2 x 6 factorial split plot design with 4 replications. Main plot treatments consisted of two post-plant water acidification treatments, randomly divided into two plots that are irrigated with either acidified or non-acidified water produced from the sulfur burner system three times per week. Sub plots consisted of six sulfur applications as subplots with 10 plants/ subplot. Soil analyses are being completed in the spring and fall yearly for all plots to provide a comparative measure for soil chemical change over time. Plant growth is assessed by visual ratings of vigor and height, as well as leaf tissue nutrient content, leaf chlorophyll levels, and multispectral aerial imagery. Fruit parameters of bud set, fruit quality (titratable acidity, firmness, mass, Brix), and yield were assessed in year 2. Preliminary data indicate soil pH trends are similar between dry S prills applied at planting and the grower standard application 8 months prior to planting. Treatments with micronized Salone did not provide soil acidification to suitable levels for blueberry. However, dry S prills at planting in combination with micronized S reduced soil pH rapidly to the target range. Acidified water helped reduce soil pH compared to non-acidified irrigation water, but does not appear to slow pH levels from raising over time. Our calculation of S to apply both pre-plant and at planting was an underestimate thus we did not reach a target pH from the initial application in most treatments. However, trends between treatments at the same rate, but applied differently support the potential of dry incorporated S at planting alone or with micronized S. This method may eliminate the need for a pre-plant waiting period that will increase blueberry productivity and decrease costs.

OBJ 2. Three un-amended field soils were collected from Washington (Quincy sands and Warden silt loams) and Oregon (Atkins sandy loam) on 2 Oct. 2019. These soil types are representative of what growers east of the Cascade Range utilize for blueberry production. Soils were divided into 5 treatments per soil type and tested for organic matter content, initial pH, and free lime content before treatment application. Our treatments include: 1) Unamended soil (control); 2) Soil + composted grape pomace (CGP); 3) Soil + CGP + elemental sulfur in micronized form (Crusade DF Micronized Wettable Sulphur WDG); 4) Soil + CGP + prilled sulfur pellets (TIGER 90CR[®]; Organic Sulfur); and 5) Soil + Leonardite. The leonardite treatment is a new addition and was added because of its potential to improve organic matter levels and is soluble in alkaline soil. We had acquired all supplies by 18 Nov. 2019. However, composted pomace meeting our requirements (i.e., not co-composted with dairy manure) was not commercially available. To overcome this, we acquired fresh pomace derived from red wine grapes from a commercial operation in Prosser, Washington. We transported it and had it composted with apple orchard wood chips (also obtained in Prosser, WA) at the WSU Puyallup Research and Extension Center. Measurements were recorded throughout the composting process and the compost was finished and ready for use by March 2020. However, the COVID pandemic required us to halt all new experiments in 2020 and obtain a no-cost extension. In 2021, the greenhouse trial with composted grape pomace is underway in the greenhouse at Washington State University Northwestern Washington Research and Extension Center. Initial soil samples as well as soil samples collected at 2, 4, and 8 weeks as well as 6 and 9 months were sent to a commercial laboratory (Soiltest, Moses Lake, WA). Samples were analyzed for pH, free lime content, carbonates, organic matter, total C and N, EC, soluble ions, and macro- and micro-nutrients. Monthly leachate data collection and measurement of leachate pH and EC have also been ongoing. After one year, live plants will be added as a bioassay and we will select the most promising treatments for scaling up into field trials in 2022.

OBJ 3 A controlled experiment was conducted in a greenhouse as planned to evaluate potential feedstocks for producing a suitable and cost-effective biochar for blueberry. Biochars were individually produced from three locally available feedstocks (apple, blueberry, and grape prunings) at different pyrolysis temperatures (350 and 700 °C) and a control (nonpyrolyzed), and were either used "as is" or were co-composted for 6 weeks with grape pomace. Each treatment was mixed at 20%, by volume, with Atkins sandy loam soil and planted in 4-L pots with "Duke" blueberry. Additional treatments included soil with no amendments or 20% compost only. The plants were watered, as needed, and fertilized weekly using a dilute solution of organic nitrogen, phosphorus, and boron fertilizers. The pH of leachate collected from the pots averaged 4.5-5.5 in each treatment, which is the recommended range for highbush blueberry; however, electrical conductivity of the leachate was 15-45% higher than recommended (< 2 dS/m) when soil was amended with biochar only. Consequently, plants grew better with co-composted biochar than with biochar alone. Overall, plants grown with co-composted apple biochar produced at 350 °C had greater leaf area and 20% to 80% more total dry weight than those grown with any other treatment, including those with no amendment or compost only. A 0.4-acre field trial was established at OSU HAREC as planned to determine the effects co-composting and placement of biochar on plant growth and early fruit production in new organic planting of highbush blueberry. Biochar was used "as is" or co-composted with grape pomace and either applied in a band or as a thin layer on the soil surface of the rows prior to shaping the planting beds. Additional treatments included apple wood chips (industry standard) and compost only, which were likewise incorporated into the planting beds. Once the treatments were established, the field was planted with 'Draper' blueberry in May 2021. Soil was sampled before and after the treatments were established and analyzed for pH, EC, nutrients, microbial community

structure, soil carbon fractions. Additional soil measurements, including water infiltration rates, hydraulic conductivity, and water holding capacity, is also underway. Leaves were collected for nutrient analysis, and canopy development is monitored using an unmanned aerial system equipped with a multispectral camera. The plants will be pruned for fruit production in 2022. OBJ 5. Templates (Excel spreadsheets) were developed for data collection in Objectives 1-3 that will be used for the cost-benefit analysis, in particular comparison of net economic benefits relative to those of the standard (baseline) practice. The templates were provided to PDs Lukas, DeVetter and co-PD Bryla to record expenses, application rates of production inputs and crop yield for each treatment combination as stated in the objectives. The completion of these templates is ongoing. OBJ 6. Assessments of current practices with Washington and Oregon growers are planned to occur in August and October 2022 and is being led by co-PI Rebecca Sero. A list of interview questions was developed with PI Lukas and co-PIs DeVetter and Hoheisel. The questions were submitted to the WSU Internal Review Board (IRB) to receive an exemption from full IRB review status. **Publications**

PROGRESS

2019/09 TO 2020/08 Target Audience: The target audience reached throughout this period consisted of regional growers. Although extension events were restricted due to the pandemic, project PD's were still able to communicate progress to stakeholders. Changes/Problems: As a result of the pandemic, research objectives 2 and 5 are delayed. Specifically, objective 2 was impacted because greenhouse entry restrictions at WSU did not allow personnel to be on-site, thus this objective must be delayed by approximately one year, as the field trial will be informed from the results of the greenhouse experiment. A no-cost extension has been filed and approved for this objective. Objective 5 was impacted because travel restrictions and group activities were suspended, meaning that in-person panels and assessments were not feasible. Growers indicated that virtual assessments over remote conferencing were not likely to be attended and postponing until in-person would be preferable. What opportunities for training and professional development has the project provided? The project has been aiming to reach blueberry producers through video programming. In-person activities are not possible due to the COVID-19 pandemic. Video programming is intended to keep stakeholders engaged in the project and to demonstrate practices for acidification methods to improve nutrient management in blueberry crops. Professional training for project personnel has included field research and extension opportunities for two graduate students and one undergraduate intern. These students have all been able to directly engage in data collection and experimental design for greenhouse and field research. How have the results been disseminated to communities of interest? At this early stage of the project, no results have been produced. We are in the establishment phase where experimental treatments are currently being administered. These steps have been disseminated through video productions. In years two and three, when results are occurring, dissemination will be through oral and poster presentations, field days, and publications. What do you plan to do during the next reporting period to accomplish the goals? In the next two years, we plan to resume grower focus groups to encourage stakeholder engagement. We will also resume in-person activities at the research field sites. As results are produced, articles will be posted on project websites, eOrganic, and conference events.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? 1. All proposed methods for this objective for year one have been completed. The OSU HAREC field site was amended with grower standard practices in November 2019, all control and experimental treatments were initiated in March 2020. Irrigation, injectors, and the water acidification sulfur burner were installed in March. The field trial was arranged in a 2 x 6 factorial split plot design with 4 replications. Main plot treatments consisted of two post-plant water acidification treatments, randomly divided into two plots that are irrigated with either acidified or non-acidified water produced from the sulfur burner system three times per week. Sub plots consisted of six sulfur applications as subplots with 10 plants/ subplot. Blueberry ('Duke') were planted on 2 Apr. 2020. Baseline soil analyses were completed on each plot to provide a comparative measure for soil chemical change over time. After blueberry plants were established, plant growth was assessed by visual ratings of vigor and height, as well as leaf tissue nutrient content, leaf chlorophyll levels, and multispectral aerial imagery. Direct soil parameters of moisture, electrical conductivity, temperature, and pH were tracked on a regular basis. The season concluded with a final set data collection completed 27 Aug. 2020, in which a full soil analysis was conducted for each plot, as well as a final set of visual ratings, leaf tissue nutrient content, leaf chlorophyll levels, and multispectral aerial imagery. 2. Three unamended field soils were collected from Washington (Quincy sands and Warden silt loams) and Oregon (Atkins sandy loam) on 2 Oct. 2019. These soil types are representative of what growers east of the Cascade Range utilize for blueberry production. Soils were divided into 5 treatments per soil type and tested for organic matter content, initial pH, and free lime content before treatment application. Our treatments include: 1) Unamended soil

(control); 2) Soil + composted grape pomace (CGP); 3) Soil + CGP + elemental sulfur in micronized form (Crusade DF Micronized Wettable Sulphur WDG); 4) Soil + CGP + prilled sulfur pellets (TIGER 90CR® Organic Sulfur); and 5) Soil + Leonardite. The leonardite treatment is a new addition and was added because of its potential to improve organic matter levels and is soluble in alkaline soil. We had acquired all supplies by 18 Nov. 2019. However, composted pomace meeting our requirements (i.e., not co-composted with dairy manure) was not commercially available. To overcome this, we acquired fresh pomace derived from red wine grapes from a commercial operation in Prosser, Washington. We transported it and had it composted with apple orchard wood chips (also obtained in Prosser, WA) at the WSU Puyallup Research and Extension Center. Measurements were recorded throughout the composting process and the compost was finished and ready for use by March 2020. However, the COVID pandemic required us to halt all new experiments. Therefore, this experiment is delayed. As of July 2020, we have started to set-up the trial now that we are at phase 2 and can resume some operations. The result of the pandemic has caused this objective to be delayed by approximately one year, as the field trial will be informed from the results of the greenhouse experiment. 3. A controlled experiment was initiated in a greenhouse to evaluate potential biochars for organic production of northern highbush blueberry. We utilized prunings from a commercial blueberry field, as well as from commercial apple orchard and wine grape vineyard, to produce the biochars. Each biochar was created at three different temperatures, including 0 (control), 350, and 700 °C, and later used "as is" or after co-composting them for 8 weeks with grape pomace (20% biochar and 80% grape pomace, by volume). The physiochemical properties of each biochar, including surface area, pH, electrical conductivity (EC), and content of ash, carbon, nitrogen, and other nutrients, were characterized using protocols established by the International Biochar Initiative. Once the composting process was finished, each biochar was mixed at 20%, by volume, with Atkins sandy loam soil (acidified beforehand to a pH of 5 using elemental sulfur) and added to 4-L pots. This resulted in a 3 × 3 × 2 experiment with 18 biochar treatments, including a combination of three pyrolysis temperatures (0 (control), 350, and 700 °C), three feedstocks (prunings of 'Duke' blueberry, 'Fuji' apple, and 'Cabernet Sauvignon' wine grape), and two levels of composting (no compost and co-composted with grape pomace). Additional treatments included "soil only" and "compost only". The pots were then planted with 'Duke' blueberry and placed in a greenhouse in a randomized complete block design with six replicates per treatment. Each pot is currently being watered, as needed, and fertilized three times per week using a dilute solution of organic fertilizer. The plants will be grown in the pots for at least three months and afterwards measured for leaf area, shoot and root dry weight, mycorrhizal colonization, and leaf nutrient concentrations. Soil samples will also be collected and analyzed for pH, EC, CEC, soil organic matter content, and Mehlich 1 nutrients. The most promising biochars will be selected for a second greenhouse trial to verify the results. The biochar that results in the most plant growth in the greenhouse will be used in a field trial. The field trial will be established at OSU HAREC and planted with 'Duke' blueberry in spring 2021. 4. Templates (Excel spreadsheets) were developed for data collection in Objectives 1-3 that will be used for the cost-benefit analysis, and provided to PDs Lukas, DeVetter and co-PD Bryla. The templates will allow investigators to record expenses, application rates of production inputs and crop yield for each treatment combination. For Objective 1, the focus of data collection is on sulfur application (cost of sulfur and labor hours) per year for each type of irrigation (with or without acidified irrigation water; crop yield in Year 3 for each treatment; irrigation system and machinery requirements; and other inputs of production related to sulfur application that are different from our baseline (i.e., 2016 enterprise budget for organic blueberries). For Objective 2, the template allows recording of data on application rates and cost of biochar, and labor hours associated with applying biochar per year for each type of application - wide or narrow band; crop yield in Year 3 for each treatment; irrigation system and machinery requirements; and other inputs of production related to soil amendment application that are different from the baseline. And lastly, for Objective 3, data collected using the template will include the per-year cost of CGP, application rates and associated labor hours in each treatment (i.e., soil incorporation before planting or application as mulch on soil surface); crop yield in Year 3 for each treatment; irrigation and machinery requirements; and other inputs of production related to CGP application that are different from the baseline. 5. This objective was not possible because of the pandemic. The first step was to organize grower groups based on region to conduct an assessment of current practices. This will be rescheduled when Oregon and Washington guidelines allow. **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 No publications reported this period.

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Is There a Role for Microbial Management in Organic Agriculture?

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Project No.	PENW-2019-03513
Agency	NIFA PENW\
Project Type	OTHER GRANTS
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Investigator(s)	Bell, T. H.; Kaye, JA, PH.; Busch, AN, KA.
Performing Institution	PENNSYLVANIA STATE UNIVERSITY, 408 Old Main, UNIVERSITY PARK, PENNSYLVANIA 16802-1505

NON-TECHNICAL SUMMARY

Many organic farmers understand that soil microorganisms are important for soil function, but are unsure how to effectively manage microbial populations. Soil microorganisms are increasingly important as farmers transition to organic agriculture, as microbial services need to replace benefits that would otherwise have been provided by chemical inputs. For example, microorganisms can transform nutrients from forms that are unavailable to plants to forms that are available to plants, which limits the need for fertilizer additions. Attempts to directly manage microorganisms are common in organic agriculture. Popular approaches include the development of compost that is expected to contain beneficial microorganisms, Korean natural farming, and the application of live microbial products obtained commercially. This latter approach in particular is growing in popularity. For example, a substantial majority of organic farmers in Ohio used microbial products in 2014, which was more than twice the proportion measured in 2009. However, microbial communities are incredibly diverse and complex, and to the farmer, invisible. This invisibility makes it hard for farmers to benchmark potential solutions, leaving them without a real framework for microbial management, and thus, no clear consensus on which approaches to apply. In this project, we will examine how soil type and farming practices interact with both passive and active microbial management, in collaboration with farmer partners, and within a long-term organic farming project at Penn State. Our Research Objectives are to: 1) Determine how location, existing management, and microbial re-seeding impact microbial colonization of soils. This will help farmers to understand the impact of including diverse plants in or around production areas on microbial diversity, and will also validate the impact of additional management treatments that they are using. 2) Link the establishment of OMRI-certified microbial products to soil traits, and determine whether on-farm conditioning can enhance product survival. Microbial products have a long history of unpredictable effectiveness, and we aim to advance understanding of where products will be effective and why. 3) Evaluate the sensitivity of soil respiration, a widely used metric for microbial health, to microbial composition. Many organic farmers pay to assess soil respiration, with the assumption that it reflects soil biological health; however, respiration readings are confounded by differences in soil characteristics. We aim to determine how sensitive this assay is to microbiological differences, and benchmark this against two other potential metrics of soil biological activity. Our Extension Objectives are to work with farmers to understand the role of soil microorganisms in organic agriculture through annual meetings and farmer conferences, and make lasting contributions to their decision-making through Extension publications that communicate our results and reveal the breadth of microbial management used by organic farmers. We believe that providing empirical guidance on

microbial management is going to help organic and transitioning farmers assess the risks and rewards of investing in this area.

OBJECTIVES

Our long-term research goals are to: Understand the role of microbial management in soils undergoing organic transition. Establish how and when to use OMRI-certified microbial products to predictably increase economic benefits to organic farmers. Determine microbial contributions to common soil health metrics. Translate and extend what we learn to empower organic farmers to manage soil microbiomes to meet their goals. Our research in this project aims to answer the following questions: How do location, existing management, and microbial re-seeding impact "natural" microbial colonization of soils? Can we enhance the predictability with which OMRI-certified microbial products establish and function in soils? To what extent does changing soil microbial composition impact a commonly used metric of soil microbial health? Our extension objectives in this project are to: Work with farmers to develop a mutual understanding of the role of soil microbes in organic agriculture through annual meetings and presentations at farmer conferences. Make lasting contributions to farmer decision-making by producing Extension publications that A) communicate our results and B) reveal the breadth of microbial management approaches that are used by farmers in our region.

APPROACH

O1: How do location, existing management, and microbial re-seeding impact "natural" microbial colonization of soils? At each of 5 participating farms, we will work with farmers to establish replicate transects of 'microbial traps' on production land that increase in distance from a bordering forest patch. This will also be done at a long-term organic field site at Penn State. To create the microbial traps, soil will initially be collected from a long-term organically managed field site at Penn State that is managed by Co-PI Kaye, to standardize the abiotic conditions that microbes will colonize. Collected soil will be sterilized, and will then receive 1 of 4 treatments: 1) no additives; 2) sterile compost; 3) incorporation of 1% forest soil from neighboring forest patch; and 4) farmer-determined treatment. Each treatment will be allocated to ~18 μm nylon mesh bags, which allow most microorganisms to enter or exit freely. Traps will be half buried before planting in Year 1 of the project, with one surface exposed to air, to allow rain and dew to act as microbial sources. Traps will be collected at 4 and 16 months post-installation, and microbiome composition assessed (high-throughput sequencing of bacterial and fungal DNA markers). The metabolic capacity of soils closest to and furthest from the forest will be assessed with the MicroResp assay. We also aim to identify the active colonizing microbial pool associated with different long-term organic cover crop treatments at Penn State. Duplicate microbial traps will be introduced to 4 replicate plots for each of 12 long-term cover crop treatments that vary in cover crop diversity. We will collect traps 4 and 16 months after burial to assess microbiome composition.

O2: Can we enhance the predictability with which OMRI-certified microbial products establish and function in soils? i) Test association between optimal pH and resource preferences of microbial products and compatibility with recipient soils. We will screen bacteria from OMRI-certified products intended for soil application, to assess tolerance to different abiotic conditions. For each product, we will determine the pH range tolerated by the organism in culture and the organism's optimal pH. Next, we will assess bacterial growth in sterilized soils collected from organic land at Penn State and from our farmer partners. Growth in each soil will be benchmarked after 2 and 4 weeks against a control in which the original soil microbiome is re-introduced. Growth will be assessed by 1) DNA extraction and quantification, and 2) respiration, using MicroResp. We will also assess resource use by each product, to determine which soil C sources are likely to support growth in soil. Based on initial screening, we will select 3 products that differ most in pH tolerance and C metabolism, and perform on-farm trials to determine how product survival relates to soil traits when other microbes are present. At each site, we will pool 10 evenly spaced subsamples of the top 15 cm of soil. A portion of the pooled soil will be analyzed for soil traits, while the rest will be added to open-top 16-qt plastic storage boxes, to serve as experimental units. At each site, we will assess survival of our three isolates, each in three replicate boxes, and will include three control boxes with no product added. In parallel, we will repeat this setup, but allow our farmer partners to incorporate a soil nutrient management treatment. We will collect soil samples from each experimental unit directly post-inoculation, and at 1 week, 1 month, and 3 months after product addition. We will extract DNA from each sample and perform qPCR to assess the persistence of the added bacteria relative to the control, using primers specific to each strain. ii) Determine whether on-farm conditioning of microbial products can increase survivability in those soils. The 'ichip' is an easily constructed device, used to cultivate previously uncultivable microorganisms. Here, we will apply a modified version of this device, not to capture bacteria, but to facilitate product adaptation to particular soils. Nine devices for each product will be buried at each participating farm pre-planting in Year 1. We aim to collect at least three intact replicates per site 12 months after installation, with others left for potential longer term sampling. After collection, we will introduce

adapted and initial products to non-sterile farm soils, and track survival at 1 week, 1 month, and 3 months post-addition as described in the previous section. We will identify the farm soil that induced the largest increase in survival, and these adapted products will be selected for metatranscriptomic comparison to the initial products. Adapted products will be introduced to microcosms containing sterile and non-sterile versions of the farm soil they were adapted to, as will the initial product. Microcosms will incubate for 2 weeks, after which we will extract RNA, which will be prepared and pooled for a single Illumina HiSeq run. These data will demonstrate how each product changed to better survive in the environment.

O3: To what extent does changing soil microbial composition impact a commonly used metric of soil microbial health? In this Objective, we will test the sensitivity of soil respiration to changes in microbial composition across soil types. In parallel, we will determine the sensitivity of two other low-cost assays of soil microbial activity. Soils for this Objective will be solicited through a collaboration with PASA, in association with the large Soil Health Benchmark Study they have run since 2016. We will collect 6 soils that vary widely in abiotic characteristics, with a focus on a broad range of soil pH and organic matter, factors known to shape soil microbial composition. Each collected soil will be split in two, with one portion sterilized to eliminate all biological content. Part of the sterile portion will be sent for physicochemical analysis, to determine soil traits after sterilization. The non-sterilized portions will be used as inoculum to reintroduce microbiomes to each sterile soil. Sterile recipient soils will be distributed to laboratory microcosms, and we will reintroduce microbiomes to each soil from the ten sources of inoculum, by burying the non-sterile source soils within nylon bags. We will allow colonization from each inoculum source for either 1 week or 16 weeks. Inoculum introductions will be timed so that nylon bags are added to one-week microcosms after fifteen weeks of colonization in the twelve-week microcosms. Following colonization, nylon bags will be removed, and microbial growth continued for eight weeks to allow communities to stabilize. DNA will be extracted from a small soil sample in each microcosm, and quantified. We will then normalize microbial biomass by adding sterile soil as needed to each sample, to ensure differences in respiration relate to composition. From extracted DNA, we will assess microbiome composition. This will allow us to assess microbial richness in each microcosm, and confirm differences in community composition based on inoculum source. Total soil respiration will be assessed using MicroResp. For total respiration, each microcosm soil will be amended with water only, and CO₂ output will be compared to a sterile control for that soil type. We will also use MicroResp to assess relative metabolism of two pools of C sources (labile vs. recalcitrant) by each microbiome in each soil. Finally, we will assess decomposition of two litter types from a long-term organic cropping system at Penn State. Corn and soybean litter will be dried and weighed, and buried separately in each microcosm soil in fine-mesh litter bags for 4 months. Decomposition will be assessed by comparing final and initial dry weights.

****Progress**** 09/01/19 to 08/31/23 ****Outputs**** Target Audience: Our target audience included farmers who use organic practices, farmers with interest in organic practices, agricultural practitioners (esp. those involved in the microbial products industry), and academics with a focus on microbial ecology and/or agricultural management. We also had specific close interactions with a number of organic and transitioning farmers in Pennsylvania, with whom we established on-farm projects. Our close interaction with farmers has ensured that our work has direct relevance to the farming community and has also allowed us to understand the wide range of microbial management practices in Pennsylvania, as well as the wide range of perceived benefits and challenges of microorganisms in agriculture. Trainees were also a target audience, and the project has provided training opportunities for undergraduates, graduates, and a postdoctoral fellow.

Changes/Problems: We made a few changes through this project, some due to COVID-related disruptions. The largest of these is that we did not ultimately perform large-scale respiration assessments in collaboration with Pasa Sustainable Agriculture, despite some initial movement on this project. Unfortunately, the logistics and personnel assignments became too challenging and we decided to put more effort and emphasis on our other project objectives. A somewhat related study was published by another group in 2020 (<https://www.nature.com/articles/s41467-020-17502-z>), which also nudged us towards pursuing other goals.

What opportunities for training and professional development has the project provided? Overall, this project has provided extensive direct training for one undergraduate student (Helen Senerchia), one graduate student (Laura Kaminsky), and one postdoctoral associate (William King). Two other graduate students also became involved, working on our deployment of microbial traps in the long-term cover crop experiments that are ongoing at Penn State (Sarah Richards and Lily Cao). Our Extension work also incorporated three other graduate students (Sarah Isbell, Mara Cloutier, Suzanne Fleishman) who were involved in developing written Extension materials. Trainees on this project were engaged in a range of original research projects, both in-field and in-lab, and were involved in communication with farmers from across the state. Additionally, Laura Kaminsky engaged heavily in farmer recruitment and communication through our dispersed on-farm project, so gained substantial experience in network building. All trainees had opportunities to develop written materials, both for the academic community and for farmer partners. Laura Kaminsky won a dissertation award for her work on this project. How have the results been disseminated to communities of interest? Results have been disseminated through farmer conferences (e.g. PASA Meeting, Stroud series), interfacing with on-farm partners, Extension articles (e.g. eOrganic), scientific conferences, and academic publications.

What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported ****Impacts**** What was accomplished under these goals?

Research Objective 1 - Showed that the pool of microbial colonizers is more limited when soils have been disturbed through added nutrients or salt accumulation (as can occur in high tunnel systems), with mostly generalist microorganisms surviving (Kaminsky et al. 2020. Environmental Microbiology). - Deployed microbial traps along farm transects and showed that our ability to detect differences by location and land use type is substantially increased through the use of microbial traps that focus on collection of colonizing microbes (King et al. 2022. ISME Communications) - Deployment of corn litter in litter bags across the Cover Crop Cocktail plots appeared to show limited differences in decomposition based on plot type. - Demonstrated that rediversification of microbiomes in soil can restore at least some functions of interest, such as nitrogen cycling, with corresponding changes in functional gene composition (King et al. 2023. Environmental Microbiome). - Deployed microbial traps throughout a long-term cover crop experiment, which we sampled over two years. The biggest observed difference in microbial colonizer pools was between fallow and cover crop inclusion, although other differences were observed between cover crop types when grouped at broader levels (Richards et al. In prep). - Showed that very small-scale differences in location across root systems impact microbial function and root metabolites. This further supports conclusions from this project that small-scale differences in management may have important impacts on microbial processes. Data from that were funded by EMSL, but postdoc time to write and analyze those complementary data was funded by this project. (King et al. 2023. Plant, Cell, and Environment)

Research Objective 2 - Using two commonly sold and OMRI-certified microbial products that perform a clear and known function in soils (Actinovate and MegaPhos), we developed systems for in situ adaptation of microbial products and seeded these with the targeted products. These were deployed across the farms of on-farm partners and collected after 3, 10, and 24 months of in-field conditioning. We observed both generalized and site-specific evolution, suggesting that farm-specific adaptation of microbial products may be possible (Kaminsky et al. In prep). - Developed primers that specifically detect the bacterial species in MegaPhos, allowing us to assess its survival in experimental soils and evaluate in-soil survival (Kaminsky et al. 2021. Applied Soil Ecology). - Showed soil-specific adaptation of microbes that changed through time; however, the most substantial genomic shifts were related to in-culture growth, an unavoidable part of the microbial product pipeline. In-culture growth especially had a major influence on the in-soil survival of microbial products (Kaminsky et al. In prep).

Research Objective 3 - In collaboration with Franklin Egan and Sara Nawa from PASA, we collected soils from their Soil Health Benchmarks project, which were targeted for use in this Objective. COVID restrictions impaired our ability to move ahead on this Objective in a number of ways. We ultimately moved away from these project objectives to focus on more promising avenues.

Extension Objective 1 - Developed and maintained an on-farm network of farmers for this project. Provided them with sequencing and interpretation of soils from their farms, including detailed factsheets developed by L Kaminsky. - In preparing for Res. Obj. 2, L Kaminsky performed a screening of 9 commonly sold microbial products for organic farmers and found that four did not even contain the marketed organism. Although not a robust and replicated study, we have used this as a point of caution in presentations to farmers about the potential of microbial products. - Presentations at PASA annual meeting as well as other soil health series.

Extension Objective 2 - We published two written products for eOrganic, focused on assessment and management of in-soil microbes. - Another article was published through Penn State Extension.

Publications **Progress** 09/01/21 to 08/31/22 **Outputs**

Target Audience: Our target audience includes farmers who use organic practices, farmers with interest in organic practices, agricultural practitioners (esp. those involved in the microbial products industry), and academics with a focus on microbial ecology and/or agricultural management. We also have specific close interactions with a number of organic and transitioning farmers in Pennsylvania, with whom we established on-farm projects. Our close interaction with farmers has ensured that our work has direct relevance to the farming community and has also allowed us to understand the wide range of microbial management practices in Pennsylvania, as well as the wide range of perceived benefits and challenges of microorganisms in agriculture. The project has provided training opportunities for undergraduates, graduates, and a postdoctoral fellow.

Changes/Problems: We chose to invest more heavily in research objectives 1 and 2, due to promising results and the goals of the people we had available to the project. We decided not to further pursue research objective 3 as our original timeline and workforce was disrupted too heavily by COVID. PI Bell has moved to a new position at the University of Toronto, so this grant is now managed by previous Co-PI Jason Kaye. What opportunities for training and professional development has the project provided? Overall, this project has provided extensive direct training for one undergraduate student (Helen Senerchia), one graduate student (Laura Kaminsky), and one postdoctoral associate (William King). In the past year, two other graduate students have become involved, working on our deployment of microbial traps in the long-term cover crop experiments that are ongoing at Penn State (Sarah Richards and Lily Cao). Our ongoing Extension work has also incorporated three other graduate students (Sarah Isbell, Mara Cloutier, Suzanne Fleishman) who were involved in developing written Extension materials. In the past year: Laura Kaminsky: Heavily involved in both the research and Extension aspects of this project. Laura has helped in recruiting and maintaining our on-farm network, and connected with these partners multiple times through the year, providing them with data-based factsheets. She has also developed and given Extension presentations on microbial products (Ext. Obj. 1 and 2). Leads the project related to on-farm conditioning of

microbial products as well as microbial survival under different soil conditions and has performed extensive work in this area, resulting in one publication and a thesis award in the past year (Res. Obj. 2). William King: Heavily involved in developing projects related to assessing microbial recolonization in soils (Res. Obj. 1). Has published multiple articles on this in the past year, while working in his new position at Cornell, and showed that restoring microbial function through microbiome reintroduction is possible. Sarah Richards and Lily Cao: Collection and analysis of data related to microbial trap deployment in a long-term cover crop experiment at Penn State (Res. Obj. 1). Sarah Isbell, Mara Cloutier, Suzanne Fleishman: Publication of Extension article on microbial management that was previously not reported. How have the results been disseminated to communities of interest? Results have been disseminated through farmer events (e.g. Farming for Success Field Day), Extension publications, and academic papers. What do you plan to do during the next reporting period to accomplish the goals? Res Obj. 1 - Complete data analysis and publication of microbial traps in long-term cover crop project. Res. Obj. 2 - Complete two publications related to microbial adaptation under different soil and culture conditions. Res. Obj. 3 - We have decided not to pursue this objective further. Ext Obj. 1 - Nothing planned, but will consider invitations to speak at farmer-facing events. Ext. Obj. 2 - Nothing planned, but will consider opportunities for Extension papers if there is student interest. ****Impacts**** What was accomplished under these goals? Res Obj. 1: How do location, existing management, and microbial re-seeding impact "natural" microbial colonization of soils? Microbial trap transect project was completed and published, showing variability in active microbial colonizers across land use types, but also at very local scales. We performed a controlled experiment of microbiome reintroduction, building on previous work that had suggested this could not "rescue" microbial function in soils. With some technical adjustments in our design (mostly, choice of soils, microbial dilutions, and incubation time), we show the opposite, that certain functions can be rescued through microbiome introductions. We now have two seasons of data on the impact of cover crop mixtures on active microbial colonizers. These are currently being analyzed and we expect a publication in the coming year. Res. Obj. 2: Can we enhance the predictability with which OMRI-certified microbial products establish and function in soils? All data has been collected and much of the data has been analyzed. LM Kaminsky won a Penn State dissertation award for this work and defended her thesis at the end of 2022. She showed soil-specific adaptation of microbes that changed through time; however, the most substantial genomic shifts were related to in-culture growth, an unavoidable part of the microbial product pipeline. LM Kaminsky published a paper on the primers used to assess in-soil survival and showed differential survival of introduced microbes across soil type. We expect two other papers to be published in the next year...these are close to completion, as they were both included in LM Kaminsky's thesis. Res. Obj. 3: To what extent does changing soil microbial composition impact a commonly used metric of soil microbial health? - After consultation with PASA, we opted to not pursue this objective further and to concentrate our effort on our other objectives. Ext. Obj. 1: Work with farmers to develop a mutual understanding of the role of soil microbes in organic agriculture through annual meetings and presentations at farmer conferences. LM Kaminsky presented at the Farming for Success field day hosted by Penn State, speaking on the topic of microbial products. Ext. Obj. 2: Make lasting contributions to farmer decision-making by producing Extension publications that A) communicate our results and B) reveal the breadth of microbial management approaches that are used by farmers in our region. We added one additional Extension publication (not previously reported), summarizing our previous contributions on this topic for farmers. ****Publications**** - Type: Journal Articles Status: Published Year Published: 2023 Citation: King WL, Richards SC, Kaminsky LM, Bradley B, Kaye J, Bell TH. 2023. Leveraging microbiome rediversification for the ecological rescue of soil function. *Environmental Microbiome* 18: 7. - Type: Other Status: Published Year Published: 2021 Citation: Borrelli K, Bell TH, Isbell S, Fleishman S, Kaminsky L, Cloutier M. 2021. Understanding and Managing Soil Microbes. *Penn State Extension* <<https://extension.psu.edu/understanding-and-managing-soil-microbes>> - Type: Other Status: Other Year Published: 2022 Citation: Kaminsky LM. 2022. Invited speaker on microbial products. Farming for Success Field Day, hosted by Penn State - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Bell TH. 2022. How directed evolution in complex environments can reshape bacterial niche breadth. *Canadian Society of Microbiologists Conference*, Guelph, ON, Canada. - Type: Journal Articles Status: Published Year Published: 2021 Citation: King W, Kaminsky LM, Gannett M, Thompson G, Kao-Kniffin J, Bell TH. 2021. Soil salinization accelerates microbiome stabilization in iterative selections for plant performance. *New Phytologist* 234: 2101-2110. - Type: Journal Articles Status: Published Year Published: 2022 Citation: King WL, Kaminsky LM, Richards SC, Bradley BA, Kaye JP, Bell TH. 2022. Farm-scale differentiation of active microbial colonizers. *ISME Communications* 2: 39. - Type: Journal Articles Status: Published Year Published: 2022 Citation: Kaminsky LM, Bell TH. 2022. Novel primers for quantification of *Priestia megaterium* populations in soil using qPCR. *Applied Soil Ecology* 180: 104628. ****Progress**** 09/01/20 to 08/31/21 ****Outputs**** Target Audience: Our target audience includes farmers who use organic practices, farmers with interest in organic practices, agricultural practitioners (esp. those involved in the microbial products industry), and academics with a focus on microbial ecology and/or agricultural management. We also have specific close interactions with a number of organic and transitioning farmers in Pennsylvania, with whom we have established on-farm projects. Our close interaction with farmers ensures that our work has direct relevance to the farming community and has

also allowed us to understand the wide range of microbial management practices in Pennsylvania, as well as the wide range of perceived benefits and challenges of microorganisms in agriculture. The project also provides training opportunities for undergraduates, graduates, and a postdoctoral fellow. Changes/Problems:- We are just getting truly back to full capacity and re-evaluating where we are. RO3 has stayed on the backburner as we have prioritized other work. - We may need to look at recruiting changes, as the postdoc on the project may have other career opportunities starting in January. What opportunities for training and professional development has the project provided? This project has provided direct training for one undergraduate student (Helen Senerchia), one graduate student (Laura Kaminsky), and one postdoctoral associate (William King). Our ongoing Extension work has also incorporated three other graduate students so far (Sarah Isbell, Mara Cloutier, Suzanne Fleishman) who were involved in developing written Extension materials. Helen Senerchia: Continued to develop our farmer survey, but has now moved to another position. Looking to move this project to other personnel.(Ext. Obj. 2). Laura Kaminsky: Heavily involved in both the research and Extension aspects of this project. Laura has helped me in recruiting our on-farm network, and connects with these partners multiple times through the year. In addition to establishing research projects on their farms, she has taken samples for them which she then processed for sequencing and/or PLFA analysis. She translated those data into factsheets for the farmers to help them understand the outputs, as well as providing annual project updates (Ext. Obj. 1). Has also contributed to Extension presentations and is developing written and video-based Extension materials (Ext. Obj. 1 and 2). Leads the project related to on-farm conditioning of microbial products as well as microbial survival under different soil conditions (Res. Obj. 2). Has completed and published a project that looks at how soil conditions impact natural microbial recolonization (Res. Obj. 1). William King: Heavily involved in developing projects related to assessing microbial recolonization in soils (Res. Obj. 1). Has developed various on-farm assessment systems to assess recolonization of soils and litter, including the ability to determine how the latter impacts litter decomposition. Further, the litter-based project is nested within the long-term Cover Crop Cocktail project at Penn State, which also helps in building a larger interdisciplinary knowledge base around cover cropping in organic systems (Res. Obj. 1). Published an article focused on factors impacting in-field microbial product success (Res. Obj. 2). Suzanne Fleishman, Mara Cloutier, Sarah Isbell: Contributing to the writing of two articles published in eOrganic. How have the results been disseminated to communities of interest? Results have been disseminated through farmer conferences (e.g. PASA Meeting, Stroud series), Extension articles (e.g. eOrganic), and academic publications. What do you plan to do during the next reporting period to accomplish the goals? Res Obj. 1 - Analysis of sequences from microbial re-introduction project and corn decomposition project. - Submission of manuscript focused on microbial recolonization across transects. - Deployment, sequencing, and analysis of microbial traps in cover crop treatments, in collaboration with a colleague who will look at changes in root composition at the same sites. Res. Obj. 2 - Continued sequence analysis of adapted MegaPhos and Actinovate strains, including publication of comparison between whole genome, 100-isolate mix, and metagenome sequencing approaches. - Complete conditioning of MegaPhos to multiple physical soil environments and performing sequencing to assess adaptation. - Complete publication of in situ adaptation method. Res. Obj. 3 - Re-assess approach for this objective. Potentially advance as originally planned, but will discuss with colleagues who may have related objectives to see if we can do this in a higher impact way. Ext Obj. 1 - Present at upcoming Keystone Crop and Soils Conference and plan for at least one other oral presentation. - Continued discussion/collaboration with on-farm partners. Ext. Obj. 2 - Focus on completing and deploying farmer survey. ****Impacts**** What was accomplished under these goals? Res Obj. 1: How do location, existing management, and microbial re-seeding impact "natural" microbial colonization of soils? - Microbial trap transects that were set up by W King had DNA extracted, bacterial and fungal communities sequenced, and many analyses performed. We have a working draft of this paper and these results are guiding our upcoming work. - Corn litter deployed in cover crop treatments was assessed for decomposition and DNA was extracted and sequenced. Analyses for differences in microbial composition by treatment are ongoing. - For controlled experiment, looking at microbial re-introductions, DNA is extracted and sequenced and accessory analyses (N mineralization and enzyme assays) have been performed. Analyses are ongoing. - We are deploying soil microbial traps throughout the long-term cover crop treatments to look at how cover crops influence active microbial pools. A collaborator is helping us to compare these data to root growth, to determine how these two metrics relate in soil. Res. Obj. 2: Can we enhance the predictability with which OMRI-certified microbial products establish and function in soils? - Additional samplings of field-deployed microbial adaptation devices plus additional sequencing - Have performed numerous genome-level analyses on initial MegaPhos sequencing and are sequencing for Actinovate now. Identified numerous mutant genes, some of which appear to be involved in nutrient acquisition. - It appears the soil conditions may be more important than deployment location, so we are now modifying the incubation soil type to determine how that impacts product adaptation. Res. Obj. 3: To what extent does changing soil microbial composition impact a commonly used metric of soil microbial health? - Due to various COVID challenges and interesting results from the other research aims, we have not made substantial progress on this aim in the past year. We are evaluating the best path forward. Ext. Obj. 1: Work with farmers to develop a mutual understanding of the role of soil microbes in organic agriculture through annual meetings and presentations at farmer

conferences. - Continue to manage an on-farm network of farmers for this project. Provide them with annual project updates, as well as in-person interaction. - Presentations at PASA annual meeting as well as two other soil health series. Ext. Obj. 2: Make lasting contributions to farmer decision-making by producing Extension publications that A) communicate our results and B) reveal the breadth of microbial management approaches that are used by farmers in our region. - Two publications in eOrganic, focused on demystifying soil microbes and talking about the potential for microbial management in organic ag. **Publications** - Type: Other Status: Published Year Published: 2021 Citation: Kaminsky L, Cloutier M, Fleishman S, Isbell S, Borrelli K, Bell TH. 2021. Soil microbes in organic crop production systems 101. eOrganic <<http://eorganic.org/node/34601>>. - Type: Other Status: Published Year Published: 2021 Citation: Isbell S, Fleishman S, Cloutier M, Kaminsky L, Borrelli K, Bell TH. 2021. Management of soil microbes on organic farms. eOrganic <<http://eorganic.org/node/34646>>. - Type: Journal Articles Status: Published Year Published: 2021 Citation: King W, Bell TH. 2021. Can dispersal be leveraged to improve microbial inoculant success? Trends in Biotechnology <<https://doi.org/10.1016/j.tibtech.2021.0>>. - Type: Conference Papers and Presentations Status: Other Year Published: 2021 Citation: Bell TH, Kaminsky LM, Richards S. 2021. The present and future of boosting soil health through microbial management. Stroud Water Research Center, Avondale, PA, USA. - Type: Conference Papers and Presentations Status: Other Year Published: 2021 Citation: Bell TH. 2021. Potential for management in the soil microbiome. Crops Conference Series. - Type: Conference Papers and Presentations Status: Other Year Published: 2021 Citation: Bell TH and Kaminsky LM. 2021. Harnessing soil biology for healthy crops. Pasa Sustainable Agriculture Conference, virtual.

PROGRESS

2019/09 TO 2020/08 Target Audience: Our target audience includes farmers who use organic practices, farmers with interest in organic practices, agricultural practitioners (esp. those involved in the microbial products industry), and academics with a focus on microbial ecology and/or agricultural management. We also have specific close interactions with a number of organic and transitioning farmers in Pennsylvania, with whom we have established on-farm projects. Our close interaction with farmers ensures that our work has direct relevance to the farming community and has also allowed us to understand the wide range of microbial management practices in Pennsylvania, as well as the wide range of perceived benefits and challenges of microorganisms in agriculture. The project also provides training opportunities, for undergraduates, graduates, and a postdoctoral fellow.

Changes/Problems:- As mentioned, we could not initiate microbial recolonization transects with our on-farm partners due to COVID restrictions. Depending on our results from initiating this project at the PSU farm, we will decide on the value of pursuing this in the coming year. - Delay in initiating controlled experiments for Res. Obj. 3, again due to COVID restrictions on lab access. Expect to initiate these in the coming year. - Sequencing services have been incredibly slow this year. We hope this picks up in the next few months, or we could have unexpected delays in data generation for the project. What opportunities for training and professional development has the project provided? This project has provided direct training for one undergraduate student (Helen Senerchia), one graduate student (Laura Kaminsky), and one postdoctoral associate (William King). Our ongoing Extension work has also incorporated three other graduate students so far (Sarah Isbell, Mara Cloutier, Suzanne Fleishman) who have been involved in developing written Extension materials. Helen Senerchia: Has been involved in putting together our survey on microbial management that we hope to distribute to farmers next year. Helen was involved in creating questions, organizing the survey, transcribing our survey to RedCap, and working through IRB approvals (Ext. Obj. 2). Laura Kaminsky: Heavily involved in both the research and Extension aspects of this project. Laura has helped me in recruiting our on-farm network, and connects with these partners multiple times through the year. In addition to establishing research projects on their farms, she has taken samples for them which she then processed for sequencing and/or PLFA analysis. She translated those data into factsheets for the farmers to help them understand the outputs (Ext. Obj. 1). Has also contributed to Extension presentations and is developing written and video-based Extension materials (Ext. Obj. 1 and 2). Leads the project related to on-farm conditioning of microbial products as well as microbial survival under different soil conditions (Res. Obj. 2). Has completed and published a project that looks at how soil conditions impact natural microbial recolonization (Res. Obj. 1). William King: Heavily involved in developing projects related to assessing microbial recolonization in soils (Res. Obj. 1). Has developed various on-farm assessment systems to assess recolonization of soils and litter, including the ability to determine how the latter impacts litter decomposition. Further, the litter-based project is nested within the long-term Cover Crop Cocktail project at Penn State, which also helps in building a larger interdisciplinary knowledge base around cover cropping in organic systems (Res. Obj. 1). Suzanne Fleishman, Mara Cloutier, Sarah Isbell: Contributing to the writing of two eOrganic articles on microbial assessment and management that we expect to submit in December 2020. How have the results been disseminated to communities of interest? Results have mostly been disseminated through a pair of farmer conferences last winter and a research publication, as well as research-focused talks. In addition, we have had direct conversations with our on-farm partners regarding project progress and results. What do you

plan to do during the next reporting period to accomplish the goals? Res Obj. 1 - Collection and sequencing of samples from field deployments. Analysis of how soil conditions and distance from forest and/or cover crop treatments influenced microbial colonization. - Functional assessment of controlled experiments to determine how microbial reintroductions influence soil function. - Assess value of on-farm deployments for this project with farmer partners. Res Obj. 2 - Analysis of sequences generated to assess microbial adaptation through on-farm conditioning. - Collection of long-term on-farm samples for this project (likely collection at 16 or 18 months). - Validation of developed qPCR probes for MegaPhos as well as testing of in-soil persistence. - Attempt to develop qPCR probes for Actinovate (this has so far been a challenge). - Determine subset of field-conditioned Actinovate samples for sequencing and assessment of on-farm adaptation. Res Obj. 3 - Expect to initiate respiration experiments with soils collected through PASA collaboration. Ext Obj. 1 - Along with L Kaminsky, I will present on our work at the PASA Conference in January. We also expect to present at 1-2 research meetings, depending on what conferences look like for next year. - Continued discussion and collaboration with on-farm partners. Ext Obj. 2 - Submission and publication of two eOrganic articles focused on microbial assessment and microbial management in organic farming systems. - Publication of L Kaminsky video on microbial assessment. - Deployment of farmer survey to assess integration and perceptions of microbial management in Pennsylvania.

2020/09 TO 2021/08 Target Audience: Our target audience includes farmers who use organic practices, farmers with interest in organic practices, agricultural practitioners (esp. those involved in the microbial products industry), and academics with a focus on microbial ecology and/or agricultural management. We also have specific close interactions with a number of organic and transitioning farmers in Pennsylvania, with whom we have established on-farm projects. Our close interaction with farmers ensures that our work has direct relevance to the farming community and has also allowed us to understand the wide range of microbial management practices in Pennsylvania, as well as the wide range of perceived benefits and challenges of microorganisms in agriculture. The project also provides training opportunities for undergraduates, graduates, and a postdoctoral fellow. Changes/Problems:- We are just getting truly back to full capacity and re-evaluating where we are. RO3 has stayed on the backburner as we have prioritized other work. - We may need to look at recruiting changes, as the postdoc on the project may have other career opportunities starting in January. What opportunities for training and professional development has the project provided? This project has provided direct training for one undergraduate student (Helen Senerchia), one graduate student (Laura Kaminsky), and one postdoctoral associate (William King). Our ongoing Extension work has also incorporated three other graduate students so far (Sarah Isbell, Mara Cloutier, Suzanne Fleishman) who were involved in developing written Extension materials. Helen Senerchia: Continued to develop our farmer survey, but has now moved to another position. Looking to move this project to other personnel. (Ext. Obj. 2). Laura Kaminsky: Heavily involved in both the research and Extension aspects of this project. Laura has helped me in recruiting our on-farm network, and connects with these partners multiple times through the year. In addition to establishing research projects on their farms, she has taken samples for them which she then processed for sequencing and/or PLFA analysis. She translated those data into factsheets for the farmers to help them understand the outputs, as well as providing annual project updates (Ext. Obj. 1). Has also contributed to Extension presentations and is developing written and video-based Extension materials (Ext. Obj. 1 and 2). Leads the project related to on-farm conditioning of microbial products as well as microbial survival under different soil conditions (Res. Obj. 2). Has completed and published a project that looks at how soil conditions impact natural microbial recolonization (Res. Obj. 1). William King: Heavily involved in developing projects related to assessing microbial recolonization in soils (Res. Obj. 1). Has developed various on-farm assessment systems to assess recolonization of soils and litter, including the ability to determine how the latter impacts litter decomposition. Further, the litter-based project is nested within the long-term Cover Crop Cocktail project at Penn State, which also helps in building a larger interdisciplinary knowledge base around cover cropping in organic systems (Res. Obj. 1). Published an article focused on factors impacting in-field microbial product success (Res. Obj. 2). Suzanne Fleishman, Mara Cloutier, Sarah Isbell: Contributing to the writing of two articles published in eOrganic. How have the results been disseminated to communities of interest? Results have been disseminated through farmer conferences (e.g. PASA Meeting, Stroud series), Extension articles (e.g. eOrganic), and academic publications. What do you plan to do during the next reporting period to accomplish the goals? Res Obj. 1 - Analysis of sequences from microbial re-introduction project and corn decomposition project. - Submission of manuscript focused on microbial recolonization across transects. - Deployment, sequencing, and analysis of microbial traps in cover crop treatments, in collaboration with a colleague who will look at changes in root composition at the same sites. Res. Obj. 2 - Continued sequence analysis of adapted MegaPhos and Actinovate strains, including publication of comparison between whole genome, 100-isolate mix, and metagenome sequencing approaches. - Complete conditioning of MegaPhos to multiple physical soil environments and performing sequencing to assess adaptation. - Complete publication of in situ adaptation method. Res. Obj. 3 - Re-assess approach for this objective. Potentially advance as originally planned, but will discuss with colleagues who may have related objectives to see if we can do this in a higher impact way. Ext Obj. 1 - Present at upcoming Keystone Crop and Soils Conference and plan for at least

one other oral presentation. - Continued discussion/collaboration with on-farm partners. Ext. Obj. 2 - Focus on completing and deploying farmer survey.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? Res Obj. 1:How do location, existing management, and microbial re-seeding impact "natural" microbial colonization of soils? - L Kaminsky published a paper looking at how soil conditions interact with microbial sources to shape microbial colonization (Kaminsky et al. 2020). This paper showed that the pool of colonizers is more limited when soils have been disturbed through added nutrients or salt accumulation (as can occur in high tunnel systems), with mostly generalist microorganisms surviving. - Initially we had planned to set up transects for assessing microbial recolonization at each on-farm location, but due to COVID restrictions early in the year, we adapted this to be performed exclusively at the PSU Russell Larson Ag Center. Microbial traps were deployed across multiple transects by W King, collected at two time points, and DNA is being extracted for future sequencing. Microbial regrowth in farm soil appears to be slower than in forest soil. - W King deployed corn litter in litter bags throughout the Cover Crop Cocktail plots at the long-term organic site at the Russell Larson Ag Center in order to assess the impact of cover crops on litter colonization and decomposition. These will be collected in December to assess decomposition and microbial composition. - W King also initiated a controlled experiment which aims to look at how microbial reintroductions influence soil functioning. This will incubate for the next ~3 months, so we expect results within the next year. Res. Obj. 2:Can we enhance the predictability with which OMRI-certified microbial products establish and function in soils? - Identified two commonly sold and OMRI-certified microbial products that contain the marketed organism and that perform a clear and known function in soils (Actinovate and MegaPhos). - Developed systems for in situ adaptation of microbial products and seeded these with the targeted products. - Systems deployed to farms of on-farm partners and two organic-certified land at Russell Larson Agricultural Center at PSU. Collected after 3 and 10 months of in-field conditioning, with 1-2 more sets of units still deployed in the field. - To assess genomic change, we sequenced the ancestral and conditioned products for MegaPhos in 3 ways: 1) single collected isolates, 2) pools of 100 isolates, and 3) soil metagenomes. We will compare these approaches to determine which is ideal for assessing in situ adaptation. - L Kaminsky has developed primers that specifically detect the MegaPhos strain, allowing her to assess its survival in experimental soils. This will allow us to perform differential survival assays over the next year to see whether on-farm conditioning impacts in-soil survival. Res. Obj. 3:To what extent does changing soil microbial composition impact a commonly used metric of soil microbial health? - In collaboration with Franklin Egan and Sara Nawa from PASA, we collected soils from their Soil Health Benchmarks project, which will be used in this Objective. COVID restrictions have impaired our ability to move ahead on this Objective in a number of ways, including lab shutdowns through June, restrictions to one person at a time now, and slow sequencing from providers. Ext. Obj. 1: Work with farmers to develop a mutual understanding of the role of soil microbes in organic agriculture through annual meetings and presentations at farmer conferences. - Developed and maintained an on-farm network of farmers for this project. Provided them with sequencing and interpretation of soils from their farms, including detailed factsheets developed by L Kaminsky. - In preparing for Res. Obj. 2, L Kaminsky performed a screening of 9 commonly sold microbial products for organic farmers and found that four did not even contain the marketed organism. Although not a robust and replicated study, we use this as a point of caution in presentations to farmers about the potential of microbial products. Ext. Obj. 2: Make lasting contributions to farmer decision-making by producing Extension publications that A) communicate our results and B) reveal the breadth of microbial management approaches that are used by farmers in our region. - We have prepared the bulk of a survey that will be used to assess microbial management strategies and perceptions in Pennsylvania. We hope to distribute this in the next year. - We have made substantial progress on two written products for eOrganic, which we expect to submit in December. - L Kaminsky developed a video explaining common metrics for microbial assessment, which she is editing for publication through Penn State Extension. **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 1. Type: Conference Papers and Presentations Status: Other Year Published: 2019 Citation: Bell, T.H., & Kaminsky, L. (2019). "Can we enhance microbial contributions to regenerative ag systems?," Regenerative Grazing From The Ground Up, Pennsylvania Association for Sustainable Agriculture, Spring Creek Farm - Wernersville, PA. 2. Type: Conference Papers and Presentations Status: Other Year Published: 2019 Citation: Bell, T.H. (2019). "The invisible and essential inhabitants of soil," PASA Advanced Farmer Gathering: Collaborating to Improve Your Workforce and Your Soil, Pennsylvania Association for Sustainable Agriculture, Harrisburg, PA. 3. Type: Conference Papers and Presentations Status: Other Year Published: 2019 Citation: Kaminsky, L., Peoples, T., & Bell, T.H. (2019). "Characterizing the primary microbial colonizers of soil," Microbiome Center Networking Event, Penn State, University Park, PA. 4. Type: Journal Articles Status: Published Year Published: 2020 Citation: Kaminsky, L., Esker, P., & Bell, T.H. (2020) Abiotic conditions outweigh microbial origin during bacterial assembly

in soils. Environmental Microbiology doi:10.1111/1462-2920.15322. 5. Type: Conference Papers and Presentations Status: Other Year Published: 2020 Citation: Bell, T.H. (2020). "Can we manipulate soil microbiomes?" Colorado State University, Fort Collins, CO (virtual).

2020/09 TO 2021/08 What was accomplished under these goals? Res Obj. 1: How do location, existing management, and microbial re-seeding impact "natural" microbial colonization of soils? - Microbial trap transects that were set up by W King had DNA extracted, bacterial and fungal communities sequenced, and many analyses performed. We have a working draft of this paper and these results are guiding our upcoming work. - Corn litter deployed in cover crop treatments was assessed for decomposition and DNA was extracted and sequenced. Analyses for differences in microbial composition by treatment are ongoing. - For controlled experiment, looking at microbial re-introductions, DNA is extracted and sequenced and accessory analyses (N mineralization and enzyme assays) have been performed. Analyses are ongoing. - We are deploying soil microbial traps throughout the long-term cover crop treatments to look at how cover crops influence active microbial pools. A collaborator is helping us to compare these data to root growth, to determine how these two metrics relate in soil. Res. Obj. 2: Can we enhance the predictability with which OMRI-certified microbial products establish and function in soils? - Additional samplings of field-deployed microbial adaptation devices plus additional sequencing - Have performed numerous genome-level analyses on initial MegaPhos sequencing and are sequencing for Actinovate now. Identified numerous mutant genes, some of which appear to be involved in nutrient acquisition. - It appears the soil conditions may be more important than deployment location, so we are now modifying the incubation soil type to determine how that impacts product adaptation. Res. Obj. 3: To what extent does changing soil microbial composition impact a commonly used metric of soil microbial health? - Due to various COVID challenges and interesting results from the other research aims, we have not made substantial progress on this aim in the past year. We are evaluating the best path forward. Ext. Obj. 1: Work with farmers to develop a mutual understanding of the role of soil microbes in organic agriculture through annual meetings and presentations at farmer conferences. - Continue to manage on-farm network of farmers for this project. Provide them with annual project updates, as well as in-person interaction. - Presentations at PASA annual meeting as well as two other soil health series. Ext. Obj. 2: Make lasting contributions to farmer decision-making by producing Extension publications that A) communicate our results and B) reveal the breadth of microbial management approaches that are used by farmers in our region. - Two publications in eOrganic, focused on demystifying soil microbes and talking about the potential for microbial management in organic ag. **PUBLICATIONS (not previously reported):**
2020/09 TO 2021/08 1. Type: Other Status: Published Year Published: 2021 Citation: Kaminsky L, Cloutier M, Fleishman S, Isbell S, Borrelli K, Bell TH. 2021. Soil microbes in organic crop production systems 101. eOrganic <http://eorganic.org/node/34601> 2. Type: Other Status: Published Year Published: 2021 Citation: Isbell S, Fleishman S, Cloutier M, Kaminsky L, Borrelli K, Bell TH. 2021. Management of soil microbes on organic farms. eOrganic <http://eorganic.org/node/34646> 3. Type: Journal Articles Status: Published Year Published: 2021 Citation: King W, Bell TH. 2021. Can dispersal be leveraged to improve microbial inoculant success? Trends in Biotechnology doi.org/10.1016/j.tibtech.2021.0. 4. Type: Conference Papers and Presentations Status: Other Year Published: 2021 Citation: Bell TH, Kaminsky LM, Richards S. 2021. The present and future of boosting soil health through microbial management. Stroud Water Research Center, Avondale, PA, USA. 5. Type: Conference Papers and Presentations Status: Other Year Published: 2021 Citation: Bell TH. 2021. Potential for management in the soil microbiome. Crops Conference Series. 6. Type: Conference Papers and Presentations Status: Other Year Published: 2021 Citation: Bell TH and Kaminsky LM. 2021. Harnessing soil biology for healthy crops. Pasa Sustainable Agriculture Conference, virtual. ** **

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Smart Tillage to Reduce N₂O Emission from Organic Agriculture

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Project No.	PENW-2019-03512
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Investigator(s)	Kemanian, A. R.
Performing Institution	PENNSYLVANIA STATE UNIVERSITY, 408 Old Main, UNIVERSITY PARK, PENNSYLVANIA 16802-1505

NON-TECHNICAL SUMMARY

The reliance of grain and forage organic production on tillage, manure and cover crops to control weeds and nutrient supply creates challenges to control nitrous oxide emission and nitrogen leaching. Our prior research shows that emissions are high when plowing legume cover crops or when these cover crops are colocated with manure and plowed in. We identified the process that causes the peak emission: denitrification driven by hypoxic conditions caused by high respiration rates. The high availability of nitrate that drives denitrification losses also causes leaching. Land transitioning to organic agriculture, as well as land managed organically or conventionally, must control these harmful N losses, a fact acknowledged by stakeholders through diverse venues. We propose smart tillage to creatively regulate the distribution and concentration of cover crop residues and manure residues in the plow layer, cutting emissions of nitrous oxide by a half during the peak emission phase - the corn phase of the rotation. This is the phase that receives the largest input of both organic nitrogen and organic carbon as cover crop or manure input. Specifically, we propose to dilute cover crops residues in a thicker soil layer before inversion tillage, seclude manure from cover crop residues by applying manure after cover crop burial, and by harvesting legume aboveground cover crop biomass when practical and when above a given threshold. We termed all these practices smart tillage. The project includes novel ways of monitoring oxygen and carbon dioxide concentration in the plow layer through imaging, which can produce potent imagery for educational activities. The experiments will be done in an ongoing organic rotational experiment that will be customized to test our hypotheses, conduct field days, and support graduate work. We propose multiple avenues to receive and provide feedback to stakeholders, including testing our smart tillage alternatives in two commercial organic farms. Reducing N losses and preserving productivity is a priority for an environmentally friendly and economically vibrant organic agriculture.

OBJECTIVES

The overall goal of this project is to combine tillage, manure and cover crop use in ways that maintain or enhance productivity and reduce nitrous oxide emissions. An ancillary goal is to reduce N leaching. Objective 1: Determine if diluting cover crop residues in a 10-cm soil before inversion by tillage reduces the subsequent N₂O emissions and leaching. Objective 2: Determine if secluding manure (shallow placement) from cover crop residues (deep placement) reduces N₂O emissions and N leaching. Objective 3: Determine if removing the aboveground

biomass of legume cover crops when it exceeds 2.5 Mg/ha (≈ 70 kg/ha of N) in spring, before flail mowing and plowing before corn planting, will reduce N₂O emission and N leaching while preserving yield. Objective 4: Measure the pattern of soil O₂ and CO₂ concentration in the business as usual treatment (i.e. cover crop + manure with inversion tillage), in the cover crops residue dilution treatment, and in the cover crop residue and manure residue seclusion treatments. Objective 5: Work with cooperating producers (on-farm research) and with other stakeholders through our Penn State Extension network to collect yield at production scale and to promote the implementation of the proposed management practices.

APPROACH

Our approach has four components: (1) measure N₂O emissions, N leaching, and corn yield in business as usual and in treatments in which placement of manure and residues is manipulated; (2) measure the same variables when legume biomass is manipulated; (3) measure with granular detail the concentration of O₂ and CO₂ in the soil profile; and (4) test our tillage-smart approaches through on-farm research, proactively seeking inputs from and disseminating our results to stakeholders through Penn State Extension and other venues. The experimental site is located at Penn State's Russell E. Larson Agricultural Research Center at Rock Spring, PA. The experiment is known as the Cover Crops Cocktail. It is an experiment established in 2012 that compares cover crop species as well as mixtures of those species. This experiment is a randomized complete block design with four replications of the rotation: corn > rye cover crop > soybean > wheat (spelt) > cover crop species and mixture treatments. An important feature for the purpose of this proposal is that corn is the crop preceded by different cover crops and by manure addition. It is a full entry experiment with three entries (one for each cash crop), and our observations will occur during the third complete rotation of these crops at this site. The field experiment has 144 main treatment plots: 12 cover crop treatments x 4 replicates x 3 entries. Each plot is 24 m x 27 m. For this research we focus on the plots having the following three cover crop treatments preceding corn: crimson clover, triticale, and three-way mixture crimson clover (2/3) and triticale (1/3), with a minor proportion of winter pea. Experiment for objectives 1-3 Plots will be split in two ($24/2 = 12$ m width). One half will remain as a control (i.e. with the current management), and the other half will be split in two halves again (i.e. $1/4$ of the plot width, $12/2 = 6$ m) to accommodate the dilution and seclusion treatments. The cover crop removal will be done in the end corner of the control or intact half of crimson clover, by carving a plot of 12 m x 6 m. Thus, we are going to deal with the original 4 blocks x 3 cover crops x 3 treatment per cover crop plot = 36 plots, plus 4 blocks x 1 cover crop with biomass removal (crimson clover) = 4 plots, rendering a total of 40 plots. The current management treatment (control or BAU) works as follows. Before corn planting, the cover crop is flail-mowed, manure added on top of it, and the ground moldboard plowed. The dilution and seclusion treatments are implemented in ways that render useful comparisons and practical treatments, but not in a full combinatorial manner (which would require an immense experimental area). In the diluted-secluded treatment, the cover crop residue will be disked-in to about 7 or 8 cm to "dilute it", and only then it will be moldboard plowed. The manure is then added to the plowed surface. In the concentrated-secluded treatment, the sequence is identical except that there is no dilution of the cover crop biomass. The aboveground biomass removal treatment is identical to the current treatment except that the aboveground biomass is removed above a certain threshold. For practical purposes, the threshold will have to be based on a fixed cutting height that we tentatively set at 25 cm. However, we will relate mass and height by measuring both quantities in small 25 cm x 25 cm quadrats in the crimson clover plots and adjust this criterion if needed. Soil gas chambers as those used in our past ORG project will be installed and used to measure N₂O emissions (1 per plot). These emissions will be measured from early spring prior to cover crop and manure incorporation, to the end of the season in the corn phase. Concurrently, soil moisture and temperature will be measured in the 0-20 cm layer using a portable soil moisture probe (Campbell Sci. Inc) and thermometer. Resin bags installed below the plow layer will be used to monitor nitrate accumulation as proxy for potential N leaching. Soil sampling (0-20 cm and 20-40 cm) will be taken once every two weeks, concurrent with N₂O emission measurements, to measure the soil nitrate concentration, a labor-intensive task. The gas sampling also provides soil respiration rates (CO₂ flux). Details on gas sampling and resin processing are provided below. Experiment for Objective 4. The 36 plots will be wired with Apogee O₂ concentration and temperature sensors. The sensors will be placed at two depths, 20-cm deep (bottom of the plow layer) and 5 cm deep. These two depths match the deeper and shallower edge of a separate VisiSens™ monitoring system for O₂ and CO₂ that we are going to install in only two treatments as explained below. The VisiSens™ provides images of the O₂ and CO₂ concentration in 15 cm x 10 cm foils (or panels). The foils will be deployed in two treatments only, current and concentrated-secluded, and in three replications. A most useful aspect of our research is that we will also have the aggregated (and likely slower-time constant) information from both the Apogee O₂ sensors and the VisiSens™ system, providing a check on the overall approach to measure the concentration of these gases in the soil. Activities for Objective 5 -- Extension Two producers, Elvin Ranck (Mifflin, PA) and Klaas Martens (Penn Yan, NY), agreed to participate in this project. The goal is to test at least one variation to the traditional cover crop + manure followed by inversion in large field plots. The dilution approach is

of interest because it does not interfere with manure application, but we will decide the best option in conjunction with the producers. We will accommodate our experimental design to the layout of their fields to incorporate blocks (5) with the producer business as usual management plus one or more variation. Each plot will be 10-m long and the width will be set to four moldboard passes. We plan to visit the producers in their farms every summer, to plan for the following year corn crop and to monitor the current management of the field. The goals are to engage producer, receive their feedback, and record changes in crop performance. Progress 09/01/22 to 08/31/23

Outputs Target Audience: Scientists working in the field of greenhouse gas emission from agriculture with emphasis in organic grain and forage production systems. Producers interested in reducing the carbon footprint. Private companies interested in commercializing carbon credits as climate commodities (new)

Changes/Problems: As stated in the prior section, the powerful Visisense instrumentation requires an immense amount of labor to be deployed in the field. We obtained images that will be useful in our communication with producers, but failed to obtain "movies" showing changes in soil oxygen concentration across the soil profile. That would have let us detect with more accuracy what needs to be changed to avoid the low oxygen boundaries that leave to nitrous oxide emissions. The system is more suitable for lab work than for field work. What opportunities for training and professional development has the project provided? Dr. Montes and Dr. Kemanian, particularly the former, successfully deployed the Visisense instrument and learn to obtain high quality images of soil oxygen concentration that very clearly show the formation and destruction of anoxic soil conditions. How have the results been disseminated to communities of interest? Dr. Kemanian made two presentations in the context of the ASCENT project, funded by the Federal Aviation Administration. One of the goals of this project is to accurately estimate the carbon intensity of sustainable aviation fuel produced from agricultural feedstock. Knowledge developed in this project is clearly of utility to address challenges in fields beyond organic agriculture. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported

Impacts What was accomplished under these goals? \Entering this section for the second time. Unfortunately, figures with oxygen concentration are not being accepted by the system.\ The Visisense O₂, pH and CO₂ optical measurement system was successfully deployed in the field. The instrument was encased in a waterproof chamber that included the camera and the auxiliary light sources. The system was powered with two solar panels and voltage transformer that charged the laptop computer that controlled the system and recorded the images. The oxygen foil sensor produced high resolution images of oxygen concentration along the soil profile. Figures showing the oxygen concentration in the soil profile with saturated water conditions and very dry soil were successfully obtained (but cannot be displayed here). A figure showing an earthworm track low-oxygen footprint coming across the profile was also obtained (an earthie, we guess?). Sensing foils for pH and CO₂ did not work well, as their signal response is to dissolved CO₂ and fluid water pH and the soil conditions never produced enough fluid water to be measured by the foil photoluminescence. The clay rocky soil in which the system was deployed presented challenges to deploy the system with sealed boundaries that prevented gas diffusion through the sides of the chamber while at the same time providing a very tight contact between the foil and the soil profile. Rocks needed to be removed which resulted in irregular patchy profile surface, that did not provide a continuous tight contact with the sensing foil attached to the flat surface of the waterproof chamber. Therefore, the visisense system did not reflected the oxygen gradient along the soil profile depth. The instrument seems to be more adequate for controlled conditions work than for field deployment. Publications Progress 09/01/19 to 08/31/23

Outputs Target Audience: Academic audience: Scientists focused on reducing greenhouse gas emissions with a focus on doing so in organic systems that rely on cover crops, manure, and tillage to manage nutrient supply. Producer / Farmer audience: organic community focused on refining nitrogen management and on balancing the tradeoff associated to nutrient management in organic systems. Changes/Problems: As stated before, dealing with Covid and establishing on farm trials was extremely challenging. The lack of response of the treatments also discouraged the implementation of some of these practices in the field. Our conclusion is that A) some trials need to move to an even more controlled setting to avoid the observed variation among blocks (more even soils or even lab conditions) and B) some trials need to be done entirely on producers fields to measure the observed variation in emissions in commercial conditions and when a given climate-smart practice is implemented. The last report also stated some challenges installing and operating the Visisens instrumentation. Wonderful instrument, but not that appropriate for the heterogeneities of the field. It is more adequate for lab work. We expected that the visuals from this instrument would serve as a communication vehicle to interact with producers. The images are great, but the labor required to obtain pictures that could be mounted in the form of a movie, are staggering. However, we think this technology will continue moving forward, and we plan to submit a manuscript including some of the college images. The image of the earthworm coming across the soil profile (described in the prior report) is rather astounding. What opportunities for training and professional development has the project provided? As stated before, Allison Koehle graduated with Master of Science degree in 2021; Farzaneh Tahriri was trained in the use of Cycles and also mentored on the importance of nitrous oxide emission in ag systems using data from this experiment. Rainey Rosemond, a new PhD student in Ecology and Elizabeth Rader, a new MS students in Meteorology, toured the field to learn about the factors affecting emissions of nitrous oxide emissions. How have the results been disseminated to communities of interest? As stated in prior reports, Allison

Koehle made a presentation of data in the American Society of Agronomy annual meeting and presented a seminar in the department of Plant Science. There is substantial dissemination of results and usage of information through every user of the model Cycles, and that includes the work by a graduate student (Curt McConnell, already graduated) that used Cycles extensively. McConnell is currently a biogeochemist working in a private company dedicated to commercialization greenhouse gas emissions reduction in agriculture. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported

Impacts What was accomplished under these goals? The award allowed us to complete a large amount of work in the field and in the laboratory. Almost 7,000 gas samples were analyzed using Gas Chromatography for N₂O, CO₂ and CH₄ concentration during the field measuring campaigns of 2021 and 2022. The measurements were used to estimate emissions from the treatments applied in this experiment. Supplementing the gas emission measurements, we obtained 6,000 soil temperature and 18,000 soil water content field measurements. In total 1,150 soil samples were analyzed for organic matter, nitrate, and ammonium. The results of the experiment were largely unexpected, and unlike prior experiments, there was substantial inter-block (replication) variability. That speaks well of the experimental design, but since the variance increased with the mean of each block, detecting statistically significant differences is more challenging. There was no effect of the tillage treatments and the cover crop combination on N₂O emissions, except for a minor effect of removing legume biomass before cover crop incorporation. This result is puzzling as we had observed on previous research differences in N₂O emissions from cover crops. Emissions tended to be higher in legume cover crops like clover, and lower in gramineous cover crops like triticale. Furthermore, the smart tillage treatments were designed to enhance or inhibit the conditions for N₂O production in the soil. Our hypothesis was that the effects of cover crop type and smart tillage were additive and therefore would have produced significant differences between treatment in N₂O emissions, i.e., cover crop and smart-tillage combinations should have suppressed emissions. The results of the ANOVA for the cumulative N₂O emissions of the experimental design with statistical model: N₂O cumulative emissions = global mean + Year + Block + CoverCrop + Tillage + CoverCrop x Tillage + B x CC x T + Error, yielded the following results. Neither cover crop, tillage, or the interaction were statistically significant. The Year and Block effect were significant, with the year and block effect of comparable magnitude (Pr of > F of 0.001) Cumulative emissions were 15.8 and 6.1 kg/ha of N in years 2021 and 2022, respectively. Emissions from clover cover crops were on average about 20% higher than those from triticale, but these differences were not statistically significant (12.3 vs 9.8 kg/ha of N on average for both years). There was however substantial differences in emissions among blocks, highlighting the well know spatial variation of nitrous oxide emissions, but also obscuring treatment effects. Emissions in some blocks were staggeringly high (almost 50 kg of N as nitrous oxide in 2021; blocks 1 and 2), while others were very subdued (blocks 3 and 4). In plain terms, the most promising management practice to control emission is reducing the mass of legume incorporated after termination of the cover crop. In prior research, we stated that additions of aboveground biomass of legumes above 2 Mg/ha seems to trigger higher emissions. Therefore, we have soft evidence indicating that reducing emissions may depend on controlling the input of nitrogen via legumes. Surprisingly, mixing residues to dilute the mass of cover crops and manure in a larger soil volume had no significant effect on emissions. We are attempting other statistical analysis more suitable to the data structure (i.e. accounting for non-additivity of block effects) and that may change these primary conclusions. The magnitude of the emissions in some blocks are definitively among the highest that we have ever measured.

Publications Type: Journal Articles Status: Published Year Published: 2023 Citation: McConnell, C.A., Rozum, R.K., Shi, Y. and Kemanian, A.R., 2023. Tradeoffs when interseeding cover crops into corn across the Chesapeake Bay watershed. *Agricultural Systems*, 209, p.103684.

Progress 09/01/20 to 08/31/21

Outputs Target Audience: That primary target was academic and included both experts interested in developing methods to measure greenhouse gases, which are critical for climate commodities verification, and experts interested in the control of nitrous oxide. Changes/Problems: Nothing Reported

What opportunities for training and professional development has the project provided? A graduate student, Allison Koehle, will graduate in May of 2022 (graduated). Koehle become an expert on the science of N₂O emissions in ag systems, and expert field and lab operator that can proficiently manage field experiments and sophisticated equipment, and an agile data analyst that use both parametric and non-parametric statistics for data analysis. How have the results been disseminated to communities of interest? Allison Koehle presented a poster with the operation of the semi-automatic chamber in Salt Lake City, at the annual ASA-CSA-SSSA meeting. The poster session was well attended. Private companies also expressed some interest in our work. What do you plan to do during the next reporting period to accomplish the goals? The experimental work will be repeated to collect two years of data, with no variation on the treatments. However, addition oxygen sensors will be added to each plot. We plan to submit a paper on methods (semi-automatic chamber) in 2022, and a paper with the results of the experiment by the end of 2023. Data on soil oxygen concentration remains to be analyzed.

Impacts What was accomplished under these goals? As planned, we measured nitrous oxide emissions and carbon dioxide emission from 40 plots growing corn in an organic experiment at Rock Spring PA. The main treatments were the preceding cover crop (triticale, red clover, or a mixture that include grasses and legumes), and the arrangement of manure and residue as incorporated in the soil. The treatments sought to reduce the concentration of animal manure and cover crops

residues at the bottom of the plow layer by either separating animal manure from cover crops or by diluting the material through the plow layer, techniques lumped under the name smart tillage. A total of 2,880 flux determinations were completed through the growing season (40 plots x 4 gas samples = 160 samples x 18 days). When accumulated through the growing season, N₂O emission for the system was high with highest emissions recorded in the legume cover crop treatments. Based on one year of data, emission with business-as-usual management was high (> 10 kg ha⁻¹ of N₂O-N during the growing season), confirming prior findings. Smart tillage techniques and removing aboveground legume biomass before corn planting did not decrease N₂O emissions, indicating the potential for future research using deeper incorporation of residues. When the cover crop was triticale emissions were the lowest (11.4 vs 6.5 kg/ha of N₂O-N) but grain yields decreased by 15% from 11 to 9.1 Mg/ha when comparing clover vs triticale. That also provides an estimate of the economic cost of reducing emissions in this system. High N₂O emissions were strongly related to the CO₂ emissions and lending credence to the hypothesis that high O₂ consumption by microbial respiration is enabling high N₂O emission by creating hypoxic but not anoxic conditions in the soil. A method to measure N₂O emissions and other gas fluxes from the soil was greatly improved through the development of an automated gas sampler. That allowed managing a large number of plots in tight time windows. The Visisens new optode sensor was set up for installation in the field in the next year. ****Publications**** - Type: Journal Articles Status: Published Year Published: 2021 Citation: Saha, D., Kaye, J.P., Bhowmik, A., Bruns, M.A., Wallace, J.M. and Kemanian, A.R., 2021. Organic fertility inputs synergistically increase denitrification-derived nitrous oxide emissions in agroecosystems. Ecological Applications, 31(7), p.e02403.

PROGRESS

2019/09 TO 2020/08 Target Audience: The target audience remains the same and includes researchers, extension educators and through on-farm work stakeholders, i.e. producers, that can implement the practices investigated in this research. Changes/Problems: Testing new soil oxygen sensor that is more expensive but likely more stable under wet conditions. It is on loan from VisiSens, the same company that produces and sells the carbon dioxide and oxygen recording device. (We already purchased the imaging equipment.) What opportunities for training and professional development has the project provided? Allison Koehle, a Master of Science student, starting working on the project and is currently testing the oxygen sensors in the lab and setting up the model Cycles. How have the results been disseminated to communities of interest? Nothing Reported What do you plan to do during the next reporting period to accomplish the goals? Except for the delay caused by Covid, we have all elements to conduct the experiment in place and we will follow the protocol described in the proposal strictly. The only added piece is testing of the oxygen sensors in the lab and the modeling with Cycles, both well underway.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? We are in the first year of the experiment and we have accomplished the following: Objectives 1, 2 and 3: (i) The plot layout was carefully defined for the cover crop and tillage treatments; (ii) manure was purchased, mixed and applied at cover crop termination or as assigned to each treatment. The implementation of the treatments was postponed due to concerns about Covid-19. Objective 4) Equipment for measuring oxygen concentration has been purchased and is being test in the lab. We are testing fast reponse / less accurate and slower response / more accurate models from Apogee. We are also testing a more expensive but smaller sensor from VisiSens. In addition, we developed an automatic sampler for the static chamber used to measure nitrous oxide, carbon dioxide, and methane accumulation in the static chambers. The reason is that the field layout requires substantial walking between plots. Time is a limitation, but also is the traffic on the plots. The new system, developed by Dr. Felipe Montes, is already operational in the lab. It uses electronically controlled valves and springs to "sample" a well mixed gas volume from chambers every 20 minutes. The system requires portable, rechargeable batteries. This innovative method will make sampling efficient and more standard. It is also safe: if a sample fails the chamber can be opened and the process completed by hand. We will publish a protocol with the method to make it available to other researchers. Restrictions due to Covid remain in place. We are planning to start our sampling campaign in Spring 2021, essentially resetting the clock one year. To compensate this delay, we started modeling the systems of interest with Cycles. A newly recruited student will complete simulations before the sampling starts. ****PUBLICATIONS (not previously reported):**** 2019/09 TO 2020/08 No publications reported this period. **** ****

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Conservation of a Multifunctional Fungus for Plant Protection in Organic Cropping Systems

Accession No.	1020531
Project No.	PENW-2019-03509
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Project Type	OTHER GRANTS
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Investigator(s)	Barbercheck, M. E.
Performing Institution	PENNSYLVANIA STATE UNIVERSITY, 408 Old Main, UNIVERSITY PARK, PENNSYLVANIA 16802-1505

NON-TECHNICAL SUMMARY

Fungal endophytes are fungi that grow in plants, with neutral or beneficial effects. Endophytes inhabit the tissues of most plants, including crop plants, and can promote crop growth and suppress insect pests and plant disease. In field experiments, we will assess the effects of soil and crop management practices on the occurrence of a naturally-occurring, beneficial insect-pathogenic and endophytic fungus, *Metarhizium robertsii*. Factors that we will examine include frequency, timing, and intensity of tillage, cover crop species, and soil characteristics. In greenhouse experiments, we will determine how competitive this soilborne fungus is with other soil organisms, and how water stress affects the ability of the fungus to form a beneficial endophytic relationship with corn plants. We will also look at the interaction of stress from feeding by an insect pest, the black cutworm, and moisture stress interact, and how this interaction affects the ability of corn to host and benefit from endophytic *Metarhizium*. Overall, we will gain a better understanding of the benefits and trade-offs of *M. robertsii* to crop plants, and how to conserve this fungus in the soil to improve crop production.

OBJECTIVES

Our long-term goal is to understand how to exploit the multiple benefits of an endemic soil fungus, *Metarhizium robertsii* (Hypocreales: Clavicipitaceae) as an insect pathogen and as a beneficial fungal endophyte in organic production systems. Our immediate research objectives are to determine: 1) the prevalence of endophytic colonization of corn grown from non-inoculated and *M. robertsii*-inoculated seed in the context of an organic reduced tillage systems experiment; 2) the relative ability of *M. robertsii* to colonize corn by seed and soil inoculation under non-competitive and competitive conditions in greenhouse tests; 3) the effects of drought and soil saturation on recruitment and establishment of endophytic *M. robertsii* by corn, and on plant growth and plant defense gene expression, and 4) the effects of the interaction of abiotic stress and biotic stress from black cutworm on corn growth and defense gene expression.

APPROACH

We will use standard aseptic technique to create single-spore isolates of *Metarhizium* spp. from sporulating cadavers of *G. mellonella* used as sentinel insects of soil from a long-term field experiment designed to assess the effects of tillage and cover cropping treatments on soil health and agronomic crop performance. In field experiments to test the effects of tillage and cover crops on the prevalence of endophytic *M. robertsii* in corn, we will manually plant *M. robertsii*-treated and non-treated corn seeds in early June into experimental plots with newly germinating corn. The date and proportion of seed emergence will be recorded. We will harvest half of the plants at V2 (~12 d after germination) and half at V4 (~25 d after germination). Root and leaf tissue from sampled plants will be evaluated for endophytic colonization in the laboratory. Timed with corn sampling, we will collect mineral soil from each replicate of each treatment plot and will use one subsample each for: 1) standard sentinel insect assays with *G. mellonella* to estimate relative prevalence of entomopathogenic fungi, 2) determination of soil electrical conductivity (EC, $\mu\text{S}/\text{cm}$) and pH; 3) determination of permanganate oxidizable carbon concentrations as an indicator of labile, biologically active soil carbon and soil moisture as gravimetric soil water content; and, 4) submission to the PSU Agricultural Analytical Services Laboratory (AASL) for analyses percent sand, silt, and clay, phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), Zinc (Zn), copper (Cu), sulfur (S), cation exchange capacity (CEC), salts, and soil organic matter by loss-on-ignition (LOI). Soil properties will be used as covariates in analyses to describe the prevalence of *Metarhizium* in soil and plants. DNA will be extracted from all *Metarhizium* isolates from plants and sentinel insect using a standard protocol. Isolates will be processed for molecular analysis by standard methods. To determine the effects of inoculation method and competition with soil biota on the degree of endophytic colonization of corn, we will conduct greenhouse experiments to compare 6 treatments each in a "competitive" and "non-competitive" environment, for a total of 12 treatments: 1) Seed inoculation by soaking in *M. robertsii* spore/ Triton X-100 solution (Trt 1); 2) Seed soaked in Triton X-100 solution (control for Trt 1); 3) Soil inoculation by application of *M. robertsii* spore/Triton X-100 solution to soil with seed soaked in Triton X-100; 4) Soil inoculation by application of *M. robertsii* spore/Triton X-100 solution to soil with seed soaked in sterile distilled water (SDW) (control for Trt 3); 5) Soil inoculation by application of *M. robertsii* spore/Triton X-100 solution to soil with dry seed (control for Trt 3); and 6) Dry, untreated seed, watered with tap water (control for Trts 1 - 5). To determine the effects of water stress on the prevalence of endophytic *Metarhizium*, we will perform a greenhouse assay to assess endophytic recruitment by corn plants under stress from deficit and excess water. Treatments will include: 1) Soil inoculation by application of *M. robertsii* spore/0.05% Triton X-100 solution, adequate moisture (Trt 1); 2) Application of 0.05% Triton X-100 solution, adequate moisture (Trt 2, control for Trt1); 3) Soil inoculation by application of *M. robertsii* spore/0.05% Triton X-100 solution, moisture deficit (Trt 3); 4) Application of 0.05% Triton X-100 solution, moisture deficit (Trt 4, control for Trt 3); 5) Soil inoculation by application of *M. robertsii* spore/Triton X-100 solution, moisture excess (Trt 5); 6) Application of Triton X-100 solution, moisture excess (Trt 6, control Trt 5). We will collect and process ~100 mg sample of corn leaf and root from each plant for analysis of gene expression in response to abiotic and biotic stress treatments. We will measure expression patterns of genes belonging to the JA, SA, ET and ABA pathways that accumulate in response to stress conditions in corn using quantitative real-time PCR (qRT-PCR). We will determine the expression pattern of MYB transcription factor, dehydration responsive element binding (DREB) proteins, responsive to drought 22 (RD22) that are involved in ABA-dependent drought stress response. We will also determine the expression pattern of the plant defense-related genes LOX1 and allene oxide synthase 1 (AOS1), ethylene responsive ACC synthase 6 (ACS6), phospholipase 1 (PLD1), maize aquaporin 1 (PIP1), and PR5. We will test the interaction of moisture stress, *M. robertsii* colonization of corn, and black cutworm in whole plant feeding assays. We will also sample ~100 mg of leaf and root tissue from each plant to assess interactions between the JA and SA pathways in response to herbivory by measuring the expression of maize protease inhibitor (MPI), ribosome inactivating protein 2 (RIP2), lipoxygenases (LOX) and oxo-phytyldienoate reductases (OPR), allene oxide synthase 1 (AOS1), phospholipase 1 (PLD1), maize aquaporin 1 (PIP1), and the pathogenesis-related protein 5 (PR5). **Progress** 09/01/19 to 08/31/23 **Outputs** Target Audience: Farmers, extension educators and other agricultural professionals, scientists, undergraduate students, graduate students, general public Changes/Problems: For objective four, we were not able to recover all of the black cutworm that were applied to plants. Therefore, we were not able to determine the effects of water and *M. robertsii* treatments on black cutworm growth. We were able to record the presence and level of feeding damage to corn and will use those values in statistical analyses. What opportunities for training and professional development has the project provided? During the reporting period, one graduate student and two undergraduate students received training associated with this project. The graduate student presented information from the project at one scientific meeting. Information to this project was presented in two undergraduate courses and one graduate course. Information from the project was presented at eight extension events. How have the results been disseminated to communities of interest? Extension presentations and written materials, scientific conference, academic courses. Barbercheck, M. 2022. Organic pest management in grains with a focus on noctuids. OGRAIN Virtual Conference, University of Wisconsin. 4 February 2022. Barbercheck, M. 2022. Better pest management through soil health. Virtual Crops Conference. 3 March 2022. 34 attendees Barbercheck, M. 2022. Soil health and IPM. Perry Co. Corn Day. 28 Feb. 2022. 64 attendees

Barbercheck, M. 2022. Endophytes for Crop Protection and Growth Promotion. Perry Corn Day. 28 Feb. 2022. 64 attendees Barbercheck, M. 11 March 22. Soil health research update. Central Susquehanna Organic Growers Network meeting. New Columbia, PA. 25 attendees. Barbercheck, M., Brasier, K. 17 March 22. A growing concern: Supporting women farmers through meeting their extension needs. Penn State Extension Update. 300 attendees Barbercheck, M. 3 August 2022. Insect Pathogens for Crop Protection and Pest Management. Lunch and Learn, SEAREC, Manheim, PA. 10 interns. Barbercheck, M., R. Hoover, J. Myers. 13 Sept. 22. Soil Health Q & A. Happy Valley vineyard and Winery. Centre Co. Conservation District Event. Peterson, H., Barbercheck, M. 2022. Impact of Water Stress on the Establishment and Persistence of Endophytic and Entomopathogenic *Metarhizium robertsii*. Eastern Branch ESA Meeting, Feb. 19-21, Philadelphia, PA Academic courses: AGECO144 Principles and Practices of Organic Agriculture, Fall 2022 and Fall 2023 INTAD Global Agricultural Systems, Fall 2022 and Fall 2023 SOIL071 Sustainability, Fall 2022 and 2023

What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported **Impacts** What was accomplished under these goals? Obj. 1: In 2020 and 2021, we inoculated maize seed with spores of *M. robertsii* and grew the plants in a certified organic corn field until V4 stage (~26 days after planting). We evaluated leaf and root samples from inoculated and uninoculated control plants for endophytic colonization by *M. robertsii*. In 2020, we detected *M. robertsii* from root tissue of 1 plant out of ~300 *M. robertsii*-treated plants. We did not detect endophytic *M. robertsii* from corn foliage. Obj. 2: Endophytic colonization of maize by soil and seed inoculation in sterilized and non-sterilized soil. We conducted a series of greenhouse experiments to compare frequency and intensity of endophytic colonization of corn inoculated with *Metarhizium robertsii* through seed inoculation and application of conidia to soil in sterile and non-sterile growth medium. For seed inoculation, we soaked seed in a *M. robertsii* spore solution (1 x 10⁸ spores/ml) for 2h. For soil inoculation, we inoculated the base of corn plants growing individually in pots at the V1 growth stage with 10 ml of spore suspension (1 x 10⁸ spores/ml). We sampled all the plants at V4 growth stage and plated 6 leaf and 6 root sections (surface-sterilized) on CTC growth medium for evaluation of endophytic colonization. The experiment was repeated three times. We detected endophytic colonization in 52 of the 141 corn plants treated with *M. robertsii* (36.9%). 27 of the endophytic 52 plants (51.9%) were colonized following seed soaking and 25 (48.1%) following soil application. Among the 52 endophytically colonized plants, we detected colonization in 7 plants (13.5%) in the non-sterile soil treatment, and 45 (86.5%) in the sterile soil treatment. Inoculation method, by soaking seed in a spore solution or applying spores to the soil, did not significantly affect the proportion of corn tissues (6 root + 6 leaf sections/plant) in which endophytic colonization was detected (seed soaking mean ± st. err. = 12.8 ± 1.24%; soil application 9.7 ± 1.16%; F_{1,316} = 1.29, P = 0.2566). Colonization rate in sterile soil (10.2 ± 0.8%) was greater (F_{1,315} = 62.9, P = 0 < 0.0001) than in non-sterile soil (1.5 ± 0.8%). There was a positive relationship between proportion of corn tissues (root + leaf sections) in which endophytic colonization was detected and corn height (r_{2adj} = 0.06, F = 10.65, P = 0.0014) and biomass (r_{2adj} = 0.09, F = 2.44, P = 0.0079), but not chlorophyll content (P = 0.6069). Obj. 3: In greenhouse experiments, corn (*Zea mays*) was grown to V3 and then assigned to three soil moisture level treatments: deficit, adequate, and excess. In each water-level treatment, plants were divided into *Metarhizium*-treated and untreated groups and inoculated with *M. robertsii*. Water stress was maintained until plants reached V7, then root and leaf tissue were assayed for endophytic growth of *M. robertsii*. Presence of *M. robertsii* in the soil was confirmed by baiting with waxworm larvae. Mean root colonization in inoculated deficit, adequate, and excess water treatments was 31 ± 1.1%, 44 ± 1.2%, and 25 ± 1%, respectively. Mean leaf colonization in uninoculated deficit, adequate, and excess water treatments was 0%, 0%, and 1.2 ± 1.2%, respectively. Linear regression showed a significant positive relationship between height and intensity of maize root colonization in the deficit water treatment (r_{2adj} = 0.091, F_{1, 52} = 6.301, P = 0.01), but not in the adequate (P = 0.451) or excess (P = 0.1714) water treatments. The relative expression of *ZmLOX1* in the jasmonic acid (JA) biosynthesis pathway was significantly greater in *M. robertsii*-inoculated than in non-inoculated plants, but water treatment had no effect. There was a significant interaction between *M. robertsii* and water treatments on foliar concentrations of JA and jasmonoyl isoleucine (JA-ILE), suggesting that water stress impacts *M. robertsii* as a modulator of plant defense. Water stress, but not inoculation with *M. robertsii*, had a significant effect on the expression of MYB (p = 0.021) and foliar concentrations of abscisic acid (p < 0.001), two signaling molecules associated with abiotic stress response. Obj. 4: We conducted greenhouse and lab-based experiments to determine the effects of endophytic *M. robertsii* on growth and defense in corn inoculated with second instar larvae of the black cutworm, *Agrotis ipsilon*. At V2, we inoculated corn plants with spores of *M. robertsii* by soil drenching. Three days after inoculation, we placed early instar black cutworms on plants and allowed them to feed for five days during which time we imposed deficit, adequate and excess water treatments. At ~21 days post-inoculation, we measured plant performance and expression of selected defense genes and phytohormone content. We also evaluated the relative prevalence of *M. robertsii* spores in the soil using sentinel baiting with *Galleria mellonella*. Among the 92 corn plants treated with *M. robertsii* and BCW, endophytic colonization was detected in 51.5%, 53.1%, and 34.5% of the corn plants in the deficit, adequate, and excess water treatments, respectively. There was no difference in intensity of colonization among water treatments (P = 0.2011). Mean intensity (proportion of root and leaf section endophytically colonized) was 13.1 ± 3.6%, 12.8 ± 3.6%, and 7.0 ± 3.7% in the deficit, adequate, and excess water

treatments, respectively. There was no effect of water treatments on the prevalence of *M. robertsii* in soil, as determined by infection rate of *G. mellonella* at the end of the experiment ($P=0.1217$). Mean intensity prevalence of *M. robertsii* in soil was $46.1\pm 6.7\%$, $59.8\pm 6.6\%$, and $58.7\pm 6.8\%$ in the deficit, adequate, and excess water treatments, respectively. The relative quantification (RQ) of plant defense-related genes, benzoxazinoid 7 and ribosome inactivating protein 2 (RIP2), differed significantly among water treatments (benzoxazinoid, $F_{2,2}=4.8917$, $P=0.0201$; RIP2, $F_{2,2}=9.1288$, $P=0.0018$) but there were no significant differences due to endophytic colonization by *M. robertsii*. The RQ of benzoxazinoid 7 was greater ($P=0.0153$) in the excess (2.89 ± 0.47), compared with the deficit (0.82 ± 0.47) water treatment, but neither were different from the adequate (1.79 ± 0.47) water treatment. The RQ of RIP2 was significantly ($F_{2,2}=9.1288$, $P=0.0018$) greater in the deficit (mean= 2.57 ± 0.44) compared to the adequate (mean= 0.92 ± 0.44 , $P=0.0040$) and excess (0.96 ± 0.44 , $P=0.0049$) water treatments. The RQ of RIP2 in the adequate and excess water treatments did not differ. There was a non-significant trend ($F_{2,2}=3.2789$, $P=0.0611$) for water treatment to affect the RQ of allene oxide synthase (AOS). The mean RQ of AOS in the deficit, adequate and excess water treatments were 16.12 ± 3.81 , 4.98 ± 3.81 , and 3.60 ± 3.81 , respectively.

****Publications**** - Type: Other Status: Published Year Published: 2022 Citation: Barbercheck, M.E., Borrelli, K. A., Wallace, J. 2022. Organic Crop Production. Part 1, Section 11. Penn State Agronomy Guide. AGRS-026 - Type: Journal Articles Status: Published Year Published: 2022 Citation: Ahmad I, Jimenez-Gasco MdM, Luthe DS, Barbercheck ME (2022) Endophytic *Metarhizium robertsii* suppresses the phytopathogen, *Cochliobolus heterostrophus* and modulates maize defenses. PLoS ONE 17(9): e0272944. <https://doi.org/10.1371/journal.pone.0272944> - Type: Journal Articles Status: Awaiting Publication Year Published: 2023 Citation: Peterson, H, Ahmad, I., Barbercheck, ME (2023) Maize response to endophytic *Metarhizium robertsii* is altered by water stress PLoS ONE: pone.0289143 (in press, to be published 27 Nov. 2023) - Type: Theses/Dissertations Status: Other Year Published: 2022 Citation: Peterson, Hannah. 2022. Water Stress Causes Context Dependent Effects In Endophytic Relationship Between *Metarhizium robertsii* And Maize. https://etda.libraries.psu.edu/?search_field=all_fields&q=Hannah+Peterson Progress 09/01/21 to 08/31/22

Outputs Target Audience: Farmers and other agricultural professionals, extension educators and specialists, scientists, graduate and undergraduate students, governmental and non-governmental agricultural agencies and organizations Changes/Problems: Nothing Reported What opportunities for training and professional development has the project provided? Information from this project was incorporated into classroom lectures and extension presentations. One graduate student attended a scientific meeting and presented results from this project. How have the results been disseminated to communities of interest? Extension presentations, What do you plan to do during the next reporting period to accomplish the goals? The experiments to meet the objectives of this research are complete. We will now focus on analysis, interpretation, and publication in scientific journals and extension outlets. Impacts What was accomplished under these goals? Obj. 1: Determine the prevalence of endophytic colonization of corn grown from non-inoculated and *M. robertsii*-inoculated seed in an organic cropping systems experiment. This experiment is complete. Data are being analyzed and a manuscript is in preparation. Obj. 2: Endophytic colonization of maize by soil and seed inoculation in sterilized and non-sterilized soil Research under this objective is complete and results were reported previously. Results will be incorporated into a manuscript in preparation. Obj. 3. Research for this objective is complete and is being incorporated into a Master's thesis. In greenhouse experiments comparing the effects of deficit, adequate, and excess soil moisture on the interaction between maize and endophytic *M. robertsii*, mean colonization of maize by *M. robertsii* in deficit, adequate, and excess water treatments was $31(\pm 1.09)\%$, $44(\pm 1.19)\%$, and $25(\pm 1.01)\%$, respectively. Among all test plants, there was a significant relationship between maize root colonization and water treatment on plant height ($p<0.001$). There was a significant positive relationship between height and maize root colonization in the deficit water treatment ($p=0.01$), but not in the adequate ($p=0.451$) or excess ($p=0.1714$) water treatments. The defense gene, *ZmLox1* was upregulated in plants that were inoculated with *M. robertsii* but were not affected by water stress. LOX genes are involved in the jasmonic acid (JA) pathway, which primarily respond to biotic stressors. Water treatment had a significant effect on relative expression of MYB genes. Plants in the deficit and adequate treatments had significantly higher relative expression of MYB compared to the excess water treatment. This is consistent with previous reports which have identified the MYB gene as a positive transcription factor for drought-related genes, making upregulation of MYB advantageous for plants during drought conditions. There was no significant difference in the phytohormones SA, cZ, GA19, GA53, and IAA content due to water treatment or inoculation with *M. robertsii*. In the deficit water treatment, JA content was significantly lower in maize inoculated with *M. robertsii* ($p=0.009$) compared to uninoculated plants. Plants that were in excess water treatment and inoculated with *M. robertsii* had higher levels of JA than uninoculated plants. The results of differing effects of soil moisture level suggest that the interaction between endophytic *M. robertsii* and maize is context-dependent. The difference in phytohormone response to *M. robertsii* colonization between water treatments demonstrates how the JA pathway functions differently under different environmental conditions, which may be in part due to the complex antagonistic and synergistic relationships between JA and other phytohormone pathways. Obj. 4. We conducted greenhouse and lab-based experiments to determine the effects of endophytic *M. robertsii*. J.F. Bisch., Rehner & Humberon growth and

defense in maize (*Zea mays* L.) infected with *C. heterostrophus*. We inoculated maize seeds with spores of *M. robertsii* and, at the 3 to 4-leaf stage, the youngest true leaf of *M. robertsii*-treated and untreated control plants with spores of *C. heterostrophus*. After 96 h, we measured maize height, above-ground biomass, endophytic colonization by *M. robertsii*, severity of SCLB, and expression of plant defense genes and phytohormone content. We recovered *M. robertsii* from 74% of plants grown from treated seed. The severity of SCLB in *M. robertsii*-treated maize plants was lower than in plants inoculated only with *C. heterostrophus*. *M. robertsii*-treated maize inoculated or not inoculated with *C. heterostrophus* showed greater height and above-ground biomass compared with untreated control plants. Height and above-ground biomass of maize co-inoculated with *M. robertsii* and *C. heterostrophus* were not different from *M. robertsii*-treated maize. *M. robertsii* modulated the expression of defense genes (*lox1*, *lox3*, endochitinase 3, *pr5*) and the phytohormone content (*cis*-zeatin, gibberellin 19, and salicylic acid) in maize inoculated with *C. heterostrophus* compared with plants not inoculated with *C. heterostrophus* and control plants. These results suggest that endophytic *M. robertsii* can promote maize growth and reduce development of SCLB, possibly by induced systemic resistance mediated by modulation of phytohormones and expression of defense and growth-related genes in maize. We conducted greenhouse and lab-based experiments to determine the effects of simultaneous stresses on endophytic colonization by *M. robertsii* and the effects of endophytic *M. robertsii* on maize defense and growth. At the 2-leaf stage, we inoculated maize (*Zea mays* L.) with spores of *M. robertsii* by soil drenching. Three days after inoculation with *M. robertsii*, we placed early instar black cutworm (*Agrotis ipsilon*) onto plants and allowed them to feed for five days during which time we simultaneously imposed deficit, adequate and excess water treatments. After ~21 days post-inoculation with *M. robertsii*, we measured maize height, above-ground biomass, relative water content of leaf tissue, endophytic colonization of leaf and root tissue, plant nutrient content, and expression of selected defense genes and phytohormone content. At the end of the experiment, we also evaluated the soil for nutrient content and estimated relative prevalence of *M. robertsii* spores using sentinel baiting with *Galleria mellonella*. Data are being analyzed and a manuscript is in preparation. Publications Type: Journal Articles Status: Accepted Year Published: 2022 Citation: Ahmad, I. M.d. M. Jimenez-Gasco, D. S. Luthe and M. E. Barbercheck. Endophytic *Metarhizium robertsii* suppresses the phytopathogen, *Cochliobolus heterostrophus* and modulates maize defenses. In press. PLoS One. Ms/ # PONE-D-22-11766. Type: Other Status: Published Year Published: 2022 Citation: Barbercheck, M.E., Borrelli, K. A., Wallace, J. 2022. Organic Crop Production. Part 1, Section 11. Penn State Agronomy Guide. AGRS-026 (revision) Type: Book Chapters Status: Published Year Published: 2021 Citation: Ahmad, I., Jimenez-Gasco, M. D. M., Barbercheck, M. (2021). The role of endophytic insect-pathogenic fungi in biotic stress management. In: Giri, B. & Varma A. (Eds). Plant Stress Biology. Springer Nature, Singapore. Type: Journal Articles Status: Published Year Published: 2021 Citation: Flonc, B., Barbercheck, M., Ahmad, I. (2021). Observations on the relationships between endophytic *Metarhizium robertsii*, Spodoptera frugiperda (Lepidoptera: Noctuidae), and maize. Pathogens, 10, 713. **Progress** 09/01/20 to 08/31/21 **Outputs** Target Audience: Graduate and undergraduate students, scientists, farmers, agricultural professionals Changes/Problems: There have been no major changes, but our research has been slowed somewhat due to restrictions related to COVID-19. What opportunities for training and professional development has the project provided? 5 undergraduate students have participated in research activities associated with this project. How have the results been disseminated to communities of interest? Information from this project has been included in academic course lectures and in extension presentations. What do you plan to do during the next reporting period to accomplish the goals? We have initiated both field-based experiments and greenhouse experiments and these are on-going. A graduate student joined the project in Fall 2020 and she will conduct research to meet project objective 3. **Impacts** What was accomplished under these goals? Obj. 1. In Summer 2020, we inoculated the seed of maize with spores of *M. robertsii* and grew the plants until V4 stage (~26 days after planting). We evaluated the plants for endophytic colonization by *M. robertsii* in leaf and root. We detected *M. robertsii* from root tissue of only 1 plant out of ~300 *M. robertsii*-treated plants. We repeated the experiment in Summer 2021 and are currently processing the samples. 80 soil samples were collected from the four tillage intensity treatments at the experimental site and are being analyzed for matric potential, gravimetric moisture, entomopathogenic organisms, pH, EC, active C, and fertility. Soil samples collected in June in corn and soybean treatments will be submitted to Cornell Soil Health for testing. A subsample of each soil sample was baited with waxmoth larvae to assess effect of treatments on prevalence of *Metarhizium*. Fungal spores from infected cadavers have been used to create isolates, which are stored at -20 until identifications can be made. Obj. 2. We collected the soil from the field and mixed with potting mix (1:1). We sterilized half of the soil medium. We used spores of *M. robertsii* to compare the degree of endophytic colonization in maize by seed vs soil inoculation. For seed inoculation, we inoculated the treated seed with spores of *M. robertsii* (1 x 10⁸ spores/ml) and control seed with 0.1 % triton X-100 for 2h. For soil inoculation, we inoculated the base of each plant at V1 growth stage with 10 ml of spore suspension (1 x 10⁸ spores/ml) and control plant with 10 ml of 0.1 % triton X-100. We sampled all the plants at V4 growth stage and plated at CTC growth media for evaluation of endophytic colonization. We recovered more frequently from soil- compared with seed-inoculated plants ($p < 0.002$; $F_{1,20} = 12.3$; $n = 22$). We recovered *M. robertsii* more frequently from the roots of soil-inoculated plants compared with seed-inoculated plants ($p < 0.03$;

F1,15=5.89; n= 17). Plant height ($p < 0.16$; F1,21=2.1; n=22) and aboveground biomass ($p < 0.69$; F1,22=0.16; n=22) of plant grown from *M. robertsii* treatment were not different between the inoculation methods. We recovered *M. robertsii* more frequently from plants grown in sterilized medium than non-sterilized medium. None of the larvae of wax worms were infected when baited in sterilized soil whereas we detected infection when larvae were baited in non-sterilized soil suggesting the presence of *Metarhizium* in the soil. Obj. 3. In greenhouse experiments, corn (*Zea mays*) was grown to the three-leaf stage and then assigned to three soil moisture level treatments: deficit, adequate, and excess. In each water-level treatment, plants were divided into *Metarhizium*-treated and untreated groups and inoculated with *M. robertsii*. Water stress was maintained until plants reached the seven-leaf stage, then root and leaf tissue were assayed for endophytic growth *M. robertsii*. Presence of *M. robertsii* in the soil was confirmed by baiting with waxworm larvae. To confirm water stress treatments, we determined the relative water content of leaf tissue of each plant at the end of the experiment. To determine the effect of treatment on plant growth, plant height, chlorophyll content, and infrared temperature of the leaves were measured daily. Dry aboveground biomass and plant and soil nutrient analysis were conducted was measured at the end of the experiment. To determine the effect of treatments on plant defense response, leaf and root sections were collected and frozen during plant harvest and transferred to a -80 degree freezer for storage until analysis. A subsample will be sent to the University of Nebraska Integrated DNA Technologies Lab for phytohormone analysis of compounds associated with metabolic pathways linked to water and herbivory stress. To determine gene expression levels we will conduct PCR on leaf and root samples with *Metarhizium*-specific primers to amplify, gene products for analysis of the effect of treatment on gene expression in the plant phytohormone defense pathway. We have conducted one replicate of this experiment, another is currently underway, and a third is planned for December 2021. Obj. 4. The first replicate of this greenhouse experiment to meet this objective was initiated in early October 2021. **Publications** - Type: Conference Papers and Presentations Status: Published Year Published: 2019 Citation: Ahmad, I., Jiménez-Gasco, M.D.M., Luthe, D.S., Shakeel S.N., & Barbercheck, M. (2019). Endophytic *Metarhizium robertsii* affects maize growth and gene expression and growth of black cutworm by eliciting plant defense. Society for Invertebrate Pathology 2019, Valencia Spain, Jul. 28-Aug.1, 2019, Invited talk. - Type: Conference Papers and Presentations Status: Other Year Published: 2020 Citation: Ahmad, I., Jiménez-Gasco, M.D.M., Luthe, D.S., & Barbercheck, M. (2020). Mighty Microbes: The tri-trophic interactions of endophytic *Metarhizium* in maize. Penn State Microbiome Center, PA, USA, Feb. 14, 2020, invited talk. - Type: Conference Papers and Presentations Status: Other Year Published: 2020 Citation: Ahmad, I., Jiménez-Gasco, M.D.M., Luthe, D.S., & Barbercheck, M. (2020). Mighty Microbes: The tri-trophic interactions of endophytic *Metarhizium* in maize. XXVIII Plant and Animal Genome, 2020, San Diego, CA, USA, Jan. 11-15, 2020, invited talk. - Type: Journal Articles Status: Published Year Published: 2021 Citation: Flonc, B., Barbercheck, M., Ahmad, I. (2021). Observations on the relationships between endophytic *Metarhizium robertsii*, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), and maize. *Pathogens*, 10, 713. - Type: Journal Articles Status: Published Year Published: 2020 Citation: Ahmad, I., Jiménez-Gasco, M. D. M., Luthe, D. S., Barbercheck, M. (2020). Systemic colonization by *Metarhizium robertsii* enhances cover crop growth. *Journal of Fungi*, 6, 64. - Type: Journal Articles Status: Published Year Published: 2020 Citation: Ahmad, I., Jiménez-Gasco, M. D. M., Luthe, D. S., Shakeel, S. N., Barbercheck, M. (2020). Endophytic *Metarhizium robertsii* enhances maize growth, suppresses insect growth and alters plant defense gene expression. *Biological Control*, 104167. - Type: Journal Articles Status: Published Year Published: 2021 Citation: Champagne, R., Wallace, J., Curran, B., and Barbercheck, M. 2021. Rotational no-till and tillage-based organic corn produce management tradeoffs in the Northeast. *Agron. J.*, 1-14 DOI: 10.1002/agj2.20823 - Type: Journal Articles Status: Published Year Published: 2021 Citation: Wallace, J.M., Barbercheck, M.E., Curran, W., Keene, C.L., Mirsky, S.B., Ryan, M. and VanGessel, M. 2021. Cover crop-based, rotational no-till (CCORNT) management tactics influence crop performance in organic transition within the Mid-Atlantic U.S. *Agron. J.* Published on-line 11 Oct 2021, <https://doi.org/10.1002/agj2.20822> - Type: Conference Papers and Presentations Status: Other Year Published: 2019 Citation: Ahmad, I., Jiménez-Gasco, M.D.M., Luthe, D.S., Shakeel S.N., & Barbercheck, M. (2019). Endophytic *Metarhizium robertsii* enhances maize growth and suppresses insect growth by eliciting plant defense. *Plant Biology* 2019, San Jose, CA, USA, Aug. 3-7, 2019, poster presentation.

PROGRESS

2019/09 TO 2020/08 Target Audience: Farmers, agricultural professionals, scientists, graduate and undergraduate students. Changes/Problems: Our progress has been slowed somewhat due to restrictions related to COVID-19. What opportunities for training and professional development has the project provided? 5 undergraduate students are conducting research associated with this project. A Master's student joined the project in Fall 2020. How have the results been disseminated to communities of interest? Information on this project has been included in an invited seminar : Ahmad, I., Jiménez-Gasco, M.D.M., Luthe, D.S., & Barbercheck, M. (2020). MightyMicrobes: The tri-trophic interactions of endophytic *Metarhizium* in maize. Penn State

Microbiome Center, PA, USA, Feb. 14, 2020, invited talk. Presentations at conferences: Ahmad, I., Jiménez-Gasco, M.D.M., Luthe, D.S., & Barbercheck, M. (2020). MightyMicrobes: The tri-trophic interactions of endophytic *Metarhizium* in maize. XXVIII Plant and Animal Genome, 2020, San Diego, CA, USA, Jan. 11-15, 2020, invited talk. What do you plan to do during the next reporting period to accomplish the goals? We have collected baseline data on prevalence of *M. robertsii* at our reserach site and are currently conducting greenhouse experimetnt to meet project objectives. We will continue these efforts during the next reporting period. A graduate student joined the project in Fall 2020 and will conduct greenhouse experiments described in Objective 3 of the project.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? We have initioatedproject experiments. We collected the soil from the field and mixed with potting mix (1:1). We sterilized half of the soil medium. We used spores of *M. robertsii* to compare the degree of endophytic colonization in maize by seed vs soil inoculation. For seed inoculation, we inoculated the treated seed with spores of *M. robertsii* (1×10^8 spores/ml) and control seed with 0.1 % triton X-100 for 2h. For soil inoculation, we inoculated the base of each plant at V1 growth stage with 10 ml of spore suspension (1×10^8 spores/ml) and control plant with 10 ml of 0.1 % triton X-100. We sampled all the plants at V4 growth stage and plated at CTC growth media for evaluation of endophytic colonization. We recovered more frequently from soil- compared with seed-inoculated plants ($p < 0.002$; $F_{1,20} = 12.3$; $n = 22$). We recovered *M. robertsii* more frequently from the roots of soil-inoculated plants compared with seed-inoculated plants ($p < 0.03$; $F_{1,15} = 5.89$; $n = 17$). Plant height ($p < 0.16$; $F_{1,21} = 2.1$; $n = 22$) and aboveground biomass ($p < 0.69$; $F_{1,22} = 0.16$; $n = 22$) of plant grown from *M. robertsii* treatment were not different between the inoculation methods. We recovered *M. robertsii* more frequently from plants grown in sterilized medium than non-sterilized medium. None of the larvae of wax worms were infected when baited in sterilized soil whereas we detected infection when larvae were baited in non-sterilized soil suggesting the presence of *Metarhizium* in the soil. We will repeat this experiment three times. We conducted a preliminary experiment to assess relative prevalence of *M. robertsii* in the soil and natural levels of endophytic colonization in corn following AWP, triticale, or canola cover crops; and to compare the prevalence of naturally-occurring endophytic *Metarhizium* in organic corn with prevalence in corn grown from *M. robertsii*-inoculated seeds planted at the same site. Neither detection in soil nor prevalence in corn was affected by the preceding cover crop or seed treatment with *M. robertsii*. Mean prevalence in soil was $24.5 \pm 3.8\%$, and mean prevalence of endophytic corn plants was $4.4 \pm 1.3\%$. In 2018, the natural prevalence of endophytic corn was infrequent but positively related to prevalence of *M. robertsii* in soil at V4 ($r_{2adj} = 0.36$, $F_{1,14} = 9.3248$, $p = 0.0085$) but not at V2. We suggest this relationship is only significant at V4 because time is needed for *M. robertsii* to colonize corn roots, and to sufficiently colonize corn tissue to allow detection of endophytic colonization through re-isolation from plant tissue. Mean prevalence of *M. robertsii* in soil across the site was lower ($F_{1,139} = 26.43$, $p < 0.0001$) in 2018 ($6.2 \pm 0.5\%$) than in the 2016 ($17.5 \pm 1.8\%$) and 2017 ($14.5 \pm 0.4\%$), and soil moisture was negatively related to the detection of *M. robertsii* in soil ($r_{2adj} = 0.123$, $p < 0.0001$). Mean soil moisture across all corn plots was $17.5 \pm 0.4\%$ in 2018, compared with $10.7 \pm 0.4\%$ and $15.4 \pm 0.4\%$ in 2016 and 2017, respectively, with all years differing from each other ($F_{2,279} = 80.9$, $p < 0.0001$). Although soil moisture is generally positively related to germination of *Metarhizium* conidia and infection of insects in the soil, conidial survival is adversely affected by wet soil. We suggest that the unusually wet weather and associated high soil moisture in 2018 contributed to low prevalence of *M. robertsii* in soil, which resulted in low prevalence of endophytic *M. robertsii* in corn plants. We plan to conduct additional assays to understand soil factors that affect *M. robertsii* in soil and in plants. **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 No publications reported this period.

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Enhancing Indigenous Soil Microflora to Facilitate Organic Strawberry Transition in the Southeastern Us

Accession No.	1020752
Project No.	TEN2019-03527
Agency	NIFA TEN\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2019-51106-30197
Proposal No.	2019-03527
Start Date	01 SEP 2019
Term Date	31 AUG 2022
Grant Amount	\$496,738
Grant Year	2019
Investigator(s)	Butler, D. M.
Performing Institution	UNIVERSITY OF TENNESSEE, 2621 MORGAN CIR, KNOXVILLE, TENNESSEE 37996-4540

NON-TECHNICAL SUMMARY

Strawberry is an important, high-value crop for small-scale growers throughout the U.S., including the warm and humid southeastern region. Growers have typically used annual, plasticulture production systems due to multiple horticultural benefits compared to perennial, matted-row production systems that are more common in cooler regions. At the same time, the annual, plasticulture system is dependent on the use of chemical soil fumigants to control soilborne diseases. In the Southeast, soil fumigation is used to control black root rot of strawberry, a disease complex caused by several fungal plant pathogens and plant parasitic nematodes. For organic growers in this region who want to use annual production systems, or conventional growers who wish to transition this familiar system to production of organic strawberry, there is a critical need for non-chemical soil disinfestation techniques that can be successfully integrated into organic production systems to reduce inoculum of soilborne pathogens and incidence of disease, enhance plant health, and improve soil biological function. This integrated research-education project has an intermediate-term goal to provide transitional and organic strawberry growers with an increased number of chemical-independent plant protection tactics and tools for disinfesting soil. This project will provide a better understanding of disease dynamics in organic strawberry systems and help to address major barriers that limit transition to organic strawberry in the southeastern U.S. The immediate and short-term goals addressed in this proposal are to better understand the use of anaerobic soil disinfestation (ASD) strategies alone, and in combination with indigenous isolates of *Trichoderma* spp. (a beneficial soil fungus) or commercial microbial biostimulants containing mycorrhizae to suppress soilborne pathogens and plant disease, and to enhance plant growth and yields. To meet these goals, we have the following four objectives: (1) Evaluate ASD amendment lignin to nitrogen ratio effects on soilborne disease suppression, indigenous *Trichoderma* population, and strawberry colonization by mycorrhizae following ASD treatment, (2) Assess impact of pre-transplant applications of indigenous *Trichoderma* isolates and commercial biostimulants containing mycorrhizal fungi post anaerobic soil disinfestation for impact on disease development and crop performance compared to ASD treatment alone, (3) Evaluate if isolates of *Trichoderma* enhance induced resistance in strawberry post-ASD treatment, and (4) Develop open-access materials for teaching soil disinfestation in organic systems at an undergraduate level.

OBJECTIVES

This is an integrated research-education project with an intermediate-term goal to provide transitional and organic strawberry growers with an increased number of chemical-independent plant protection tactics and tools for disinfesting soil, while maintaining profitable and sustainable strawberry production systems. This project will provide a better understanding of disease dynamics in organic strawberry systems and help to address major barriers that limit transition to organic strawberry in the southeastern U.S. The immediate and short-term goals addressed in this proposal are to better understand the use of anaerobic soil disinfestation (ASD) strategies alone, and in combination with indigenous isolates of *Trichoderma* spp. or commercial microbial biostimulants containing mycorrhizae to suppress soilborne pathogens and plant disease, and to enhance plant growth and yields. To meet these goals, we propose the following four objectives: 1. Evaluate ASD amendment lignin to nitrogen ratio effects on soilborne disease suppression, indigenous *Trichoderma* population, and colonization by AMF following ASD treatment, 2. Assess impact of pre-transplant applications of indigenous *Trichoderma* spp. (*T. asperellum*, *T. koningii*, *T. harzianum*) and commercial biostimulants with mycorrhizal fungi post ASD for impact on disease development and crop performance compared to ASD treatment alone, 3. Evaluate if isolates of *Trichoderma* enhance induced resistance in strawberry post-ASD treatment, and 4. Develop and disseminate open-access educational content on the use of soil disinfestation in organic and transitional strawberry systems, and provide training to undergraduate and graduate students on research in soil disinfestation.

APPROACH

Objective 1. Evaluate ASD amendment lignin to nitrogen ratio effects on soilborne disease suppression, indigenous *Trichoderma* population, and colonization by AMF following ASD treatment. Identify cover crop residue lignin to N ratio for optimal ASD amendment incorporation to reduce survival of *Fo* and *P. ultimum* compared to an untreated control. Identify soil properties during these ASD treatments (anaerobic activity, microbial respiration, and volatile fatty acids) important to effectiveness of disinfestation treatment and related to pathogen survival. Isolate, identify and quantify indigenous soil populations of *Trichoderma* species and arbuscular mycorrhizal fungi as affected by these ASD treatments. Identify the impact of these ASD treatments on strawberry crop performance, root rot incidence, and colonization by AMF fungi. Objective 2. Assess impact of pre-transplant applications of indigenous *Trichoderma* spp. (*T. asperellum*, *T. koningii*, *T. harzianum*) and commercial biostimulants with mycorrhizal fungi post ASD for impact on disease development and crop performance compared to ASD treatment alone. Evaluate the response of inoculated *Trichoderma* spp. after ASD treatment in controlled environment and field conditions. Evaluate rate of colonization by endophytic *Trichoderma* and AMF in strawberry plants and its relationship with plant growth parameters. Evaluate disease incidence and severity of *Fusarium* and *Pythium* diseases. Objective 3. Evaluate if isolates of *Trichoderma* enhance induced resistance in strawberry post-ASD treatment. Assess if plant defense responses are induced in response to *Trichoderma* spp. (*T. asperellum*, *T. koningii* or *T. harzianum*) colonization. Assess if ASD plays any role in increasing the efficiency of *Trichoderma* spp. in inducing plant defense response. Assess if plant defense responses are induced in ASD + *Trichoderma* spp. treated plants infected with *Fo* compared to the non-ASD treated plants infected with *Fo* pathogens. Objective 4. Develop and disseminate open-access educational content on the use of soil disinfestation in organic and transitional strawberry systems, and provide training to undergraduate and graduate students on research in soil disinfestation. Develop multiple short video webinars relating to soil disinfestation practices and biological controls in organic horticulture that can be used in undergraduate courses or for professional development. Using on-farm demonstrations and grower feedback, conduct a case study related to soil disinfestation in organic horticulture. Develop two course laboratory activities related to soil disinfestation in organic horticulture. Provide opportunities for graduate and undergraduate research in soil disinfestation practices. Progress 09/01/19 to 08/31/24 Outputs Target Audience: The target audience during this final year of the project has largely been the scientific community as we have worked to finalize scientific manuscripts, as well as undergraduate and graduate students through development and refinement of class lecture and lab activities. Changes/Problems: We would be remiss to not again mention the significant impact of COVID-19 on our project, which began soon after our project was initially funded. The initial restrictions and the real impacts on personnel greatly changed the trajectory of our project as initial activities were in some cases delayed as much as 12-18 months from when we had initially planned. While we were ultimately very successful in completing our objectives, we would not have been able to complete had we not been granted multiple no-cost extensions. We are extremely grateful for the extensions which allowed for the successful completion of this project, and we thank NIFA staff for their support in these extensions. What opportunities for training and professional development has the project provided? During the final project period, the primary professional development provided by the project was through support for two Ph.D. student dissertations which each included a portion of the project research. How have the results been disseminated to communities of interest? During the final reporting period, our primary focus on result dissemination has been through the

drafting, reviewing, and submission of manuscripts related to project results. We completed two manuscripts from Obj. 1 that are currently under review, a manuscript from Obj. 2 was completed as part of a Ph.D. student dissertation and is undergoing final revisions for submission to a refereed journal in early 2025. We are currently drafting a manuscript of Obj. 3 results which we expect to submit in mid-2025. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported

Impacts What was accomplished under these goals? Although our project was significantly impacted due to COVID-19 limitations that began shortly after our project was funded, we are extremely grateful for the no-cost extensions that allowed us to complete this work by the end of the extended project period. Under Obj. 1, we evaluated wheat cover crop residue as an ASD amendment with varying lignin to N ratios (lignin:N) on survival of inoculated isolates of pathogens associated with black root rot disease complex of strawberry, including *Fusarium oxysporum* (Fo), *Globisporangium sylvaticum* (Gs) and *Rhizoctonia fragariae* (Rf). Treatments included 1) vegetative stage wheat residue, 1:1 lignin:N, 2) early reproductive stage wheat residue, 2:1 lignin:N, 3) mature wheat residue, 7:1 lignin:N, 4) a wheat bran control, and 5) a non-amended anaerobic control. During the incubation period, all treated pots had highly anaerobic soil conditions, high soil biological activity, and relatively high concentrations of volatile fatty acids in soil except for the mature wheat residue and the non-amended control treatments. Wheat residue with low lignin:N ratio (1:1 or 2:1) substantially reduced populations of Fo in recovered inoculum compared to all other treatments. Similar results were observed for Gs inoculum survival with low lignin:N ratio wheat residue, although wheat bran also effectively suppressed Gs inoculum. In the case of Rf, propagules from soil were substantially reduced in all amended (ASD) pots with the lowest propagule survival observed from the wheat bran treatment only. Our results show that amendment lignin:N ratio is an important determinant of ASD treatment effectiveness as amendments with lower lignin:N ratios provided effectively a labile substrate to enhance for soil microbial activity and soil volatile fatty acid production, and microbial activity thus enhancing ASD effectiveness in controlling Fo and Gs. Under Obj. 1, we also investigated suppression of Fo by VFAs and reduced metal cations generated during soil fermentation in soil-based ASD incubation trials in which Fo-inoculated sand, sandy loam, and silty clay soils with and without lime were amended with a range of protein: carbohydrate ratios from 0.2:1 to 32:1, to initiate ASD treatments. Soil solution VFA concentrations and soil pH were measured post-ASD. Total soil solution VFA and reduced metal concentrations were negatively correlated with Fo population in sandy and sandy loam soils, and amendment protein concentration was positively correlated with soil solution VFA concentrations in sandy and sandy loam soils. In general, finer soil textures and limed soils were associated with lower Fo suppression. Our results highlight the importance of soil texture, soil pH and amendment composition in influencing ASD effectiveness. Under Obj. 2, we evaluated ASD with sorghum-sudangrass residue as the organic amendment and compared it to wheat bran. Soil treatments were ASD + wheat bran and ASD + sorghum sudangrass. Controls were wheat bran and sorghum sudangrass amendments, without ASD. Polyethylene mesh bags with 2 g Fo inocula were buried in 5-cm depth soil in pots. After 3 weeks of ASD treatment, Fo populations were assessed from the mesh bags. Strawberry plug transplants were planted in ASD and non-ASD treated soils in pots and evaluated for disease incidence and severity after 2 months. Fo populations were assessed from soil at 3 weeks after ASD treatment, and at 2 months after transplant with standard dilution plating. Plants in ASD + sorghum sudangrass -treated soil exhibited greater vigor and less disease severity in comparison to plants in soil treated with ASD + wheat bran and control plants with no ASD. We performed a metagenomics analysis on soils with wheat bran and sorghum- sudangrass and evaluated Fo populations between the two treatments. We found that ASD + sorghum-sudangrass suppressed Fo populations more than ASD + wheat bran. In field (high tunnel) studies under Obj. 2, we found limited effects of beneficial fungal inoculants (*Trichoderma* or mycorrhizae) combined with ASD treatments, although in complimentary pot studies we found that greater abundance as endophytes in strawberry root tissue for *Trichoderma* spp. and members of the order Agaricales and family Serendipitaceae was positively associated with root and/or shoot biomass while dried molasses amendments improved Serendipitaceae abundance. This highlights the importance of soil and amendment properties and root-associated fungi in influencing ASD efficacy and crop health. Under Obj. 3, we quantified expression of pathogenesis-related genes PR-10.3 and PR-10.16 extracted from strawberry plant tissues to assess if ASD treatment and/or post-ASD *Trichoderma asperellum* (Ta) transplant inoculation plays a role in inducing strawberry defense responses in soils with/without *Fusarium oxysporum* (Fo) inoculum. We found that expression of PR-10.3 was upregulated in response to Fo inoculum in non-ASD treatments, but not ASD treatments, potentially due to ASD reducing inoculum viability. Expression of PR-10.16 was downregulated in response to Ta transplant inoculation in ASD treatments, but not non-ASD treatments. Expression of PR-10.3 was downregulated following ASD treatment relative to non-ASD treatments in the non-inoculated control, Fo, Ta and Fo+Ta treatments. Expression of PR-10.16 was highly upregulated in roots in ASD compared to non-ASD in all treatments. On the whole, induction of PR-10.16 expression could be a factor contributing to increased disease tolerance of strawberry plants following ASD treatment. We are currently drafting a manuscript relating to defense response in strawberry as related to ASD treatments as part of Obj. 3, which we expect to have under review in 2025. Under Obj. 4, we developed and evaluated (as part of existing courses) lab activity modules and completed lecture modules and short videos on ASD techniques which are currently available upon request, but will also be posted

online at the beginning of 2025 (at butlerlab.utk.edu) for open-access use and adaptation by others. Publications Type: Conference Papers and Presentations Status: Published Year Published: 2024 Citation: ? Ouma, W., M. Dee, D. Butler, U. Shrestha, J. Rice, J. Littrell, and B. H. Ownley. Comparative Metagenomic Analysis of Strawberry Rhizosphere in Soil amended with Sorghum Sudangrass or Wheat Bran during Anaerobic Soil Disinfestation Plant Health. American Phytopathology Annual Meeting, July 26-31, 2024, Memphis, TN Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Littrell, J., B. H. Ownley, and D. M. Butler. 2023. Examining the biogeochemical mechanisms of *Fusarium oxysporum* suppression in anaerobic soil disinfestation. ASA, CSSA, SSSA International Annual Meeting, St. Louis, MO. Type: Other Journal Articles Status: Under Review Year Published: 2024 Citation: U. Shrestha, James Littrell, J. Hollis Rice, B.H. Ownley, and D.M. Butler. Increased Amendment Lignin to Nitrogen Ratio Reduces Efficacy of Anaerobic Soil Disinfestation for Managing Strawberry Root Rot Pathogens. Under review, Dec. 2024. Type: Other Journal Articles Status: Under Review Year Published: 2024 Citation: James Littrell, Bonnie H. Ownley, Zachariah R. Hansen, Kimberly D. Gwinn, and David M. Butler. Role of organic amendment composition and soil texture in modulating volatile fatty acids, Fe/Mn reduction, and *Fusarium oxysporum* suppression during anaerobic soil disinfestation. Under review, Dec. 2024. Progress 09/01/22 to 08/31/23 Outputs Target Audience: The target audience during this fourth year of the project has largely been students and postdoctoral associates working on the project studies. We have also increased outreach to the scientific community through abstracts and scientific presentations, to grower, extension, and NRCS communities through outreach activities and presentations, and to undergraduate and graduate students through development of class lecture and lab activities. Changes/Problems: We remain behind on our targeted timeline due to the severe impacts of COVID-19 on research activities during the first and second reporting period, but we expect to complete the project (outside of some manuscript revisions, etc.) by the end of the coming reporting period. What opportunities for training and professional development has the project provided? Multiple undergraduate and graduate students, and postdoctoral researchers participated in the project during the reporting period, providing numerous opportunities for professional development and training in research methods related to soil disinfestation in organic strawberry systems, and plant defense responses. How have the results been disseminated to communities of interest? During the reporting period multiple activities were conducted to disseminate results to communities of interest. First, multiple scientific presentations (see abstracts in reported publications) were presented to disseminate results to the broader scientific community. Second, one workshop was conducted to train farmers on soil disinfestation systems at UT Extensions New Farmer's Academy (a training program for early career farmers) at the field research site. Lastly, prototype lab modules were further evaluated with undergraduate and graduate students at the University of Tennessee, who gained exposure to research methods and current understanding of soil disinfestation in organic horticultural systems. What do you plan to do during the next reporting period to accomplish the goals? In the coming reporting period, we plan to complete manuscript preparation as part of Obj. 1, continue manuscript/dissertation preparation as part of Obj. 2, complete RNAseq analysis and a manuscript as part of Obj. 3, and finalize development of educational materials associated with Obj. 4. We expect to continue outreach to the scientific community, grower groups, extension personnel, NRCS personnel, and undergraduate/graduate students through multiple venues. Impacts What was accomplished under these goals? Although we remain behind our anticipated time line due to COVID-19 limitations shortly after our project was funded, we are grateful for the no-cost extensions that will allow us to complete this work by the end of the next reporting period. We have completed all studies under Obj. 1 and Obj. 2, and are currently analyzing data and drafting and revising manuscripts. For Obj. 3, RNA seq analysis is in progress. Data from Obj. 1-3 was presented at multiple scientific meetings (and the NIFA-OREI/ORG project director's meeting) during the reporting period (see publications reported). For Obj. 4, we further developed and evaluated lab activity modules and are working to complete lecture modules and short videos on ASD techniques and will post for public access once completed at the end of reporting period. Publications Type: Journal Articles Status: Published Year Published: 2023 Citation: First Report of Strawberry Black Root Rot Caused by *Globosporangium sylvaticum* in Tennessee, U.S.A. U. Shrestha, M.E. Dee, B.H. Ownley, and D.M. Butler. 2023. *Plant Disease* 107:9, 2890. <https://doi.org/10.1094/PDIS-01-23-0007-PDN> Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Soil Texture, Soil pH, and Amendment Protein: Carbohydrate Ratio Affect Suppression of *Fusarium Oxysporum* under Anaerobic Soil Conditions By Volatile Fatty Acids and Reduced Metal Cations. 2022. J. Littrell, U. Shrestha, B.H. Ownley and D.M. Butler. 2022 ASA-CSSA-SSSA International Annual Meeting, 6-9 November 2022, Baltimore, MD. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Cover Crop Residue Lignin to Nitrogen Ratio Is an Important Determinant of Strawberry Black Root Rot Pathogen Inoculum Survival When Cover Crops Are Used As Substrates for ASD Treatment. U. Shrestha, J.J. Littrell, J. Hollis Rice, C. Dalton, B.H. Ownley, and D.M. Butler. 2023. *HortScience* 58:S162. <https://doi.org/10.21273/HORTSCI.58.9S.S1> Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Sorghum-Sudangrass As An Organic Amendment In Anaerobic Soil Disinfestation Decreases Disease Severity And Promotes Plant Vigor. W. Ouma, M. Dee, D.M. Butler, U. Shrestha, J.H. Rice, J.J. Littrell, and B.H. Ownley. 2023. 12th International Congress of

Plant Pathology, 20-25 Aug 2023, Lyon, France. ****Progress**** 09/01/21 to 08/31/22 ****Outputs**** Target Audience: The target audience during this third year of the project has largely been students and postdoctoral associates working on the project studies. We have also begun outreach to the scientific community through abstracts and scientific presentations, to grower, extension, and NRCS communities through outreach activities and presentations, and to undergraduate and graduate students through development of class lecture and lab activities. Changes/Problems: We remain behind on our targeted timeline due to the severe impacts of COVID-19 on research activities during the first reporting period. We hope to be able to at least near project completion by the end of the coming reporting period. What opportunities for training and professional development has the project provided? Multiple undergraduate and graduate students, and a postdoctoral researcher participated in the project during the reporting period, providing numerous opportunities for professional development and training in research methods related to soil disinfestation in organic strawberry systems. How have the results been disseminated to communities of interest? During the reporting period multiple activities were conducted to disseminate results to communities of interest. First, multiple scientific presentations and a workshop (see abstracts in publications) were presented to disseminate results to the broader scientific community. Second, two workshops (a total of five presentations) were conducted to train farmers, extension personnel, and NRCS personnel on soil disinfestation systems. This included two presentations to UT Extensions New Farmer's Academy (a training program for early career farmers) at the field research site, two presentations to participants (primarily growers, extension personnel, and students) at the Organic Farming and Gardening Field Day held at the UT Organic Crops Unit (field site for Obj. 2), and one presentation to southern region extension agents on mechanisms of anaerobic soil disinfestation in small fruit production systems as part of the annual training provided by the Southern Region Small Fruits Consortium. Lastly, prototype lab modules were further evaluated with undergraduate and graduate students at the University of Tennessee, who gained exposure to research methods and current understanding of soil disinfestation in organic horticultural systems. What do you plan to do during the next reporting period to accomplish the goals? In the coming reporting period, we plan to complete manuscript preparation as part of Obj. 1, complete greenhouse/growth chamber studies associated with Obj. 2 and begin manuscript/dissertation preparation, complete the repeated field trial as part of Obj. 2, complete growth chamber and laboratory trials as part of Obj. 3, and finalize development of educational materials associated with Obj. 4. We expect to continue outreach to the scientific community, grower groups, extension personnel, NRCS personnel, and undergraduate/graduate students through multiple venues. ****Impacts**** What was accomplished under these goals? The research limitations imposed by the COVID-19 pandemic greatly eased during this reporting period, although we remain behind our anticipated time line due to those limitations shortly after our project was funded. We have completed all studies under Obj. 1 and are preparing a manuscript to submit to a refereed journal in early 2023. We completed the first set of growth chamber/greenhouse studies under Obj. 2, and will be completing the second set in winter 2023. The first data from Obj. 2 was reported in a presentation at the APS meeting in 2022. The first year of field trials was completed under Obj. 2, and the second year began in late summer/early fall 2022. For Obj. 3, multiple growth chamber trials of strawberry plant defense response to anaerobic soil disinfestation were completed and RNA extraction for RNA seq analysis is in progress. The first data from Obj. 3 was presented at the ASHS meeting in 2022. For Obj. 4, we further developed and evaluated lab activity modules from the last reporting period. We will be completing lecture modules and short videos on ASD techniques and will post for public access once completed at the end of reporting period. ****Publications**** - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Shrestha, U., J. Littrell, S. Piya, J.H. Rice, B.H. Ownley, T. Hewezi, D.M. Butler. 2022. Expression Profiling of Pathogenesis-Related Protein-10 (PR10) Genes in Strawberry in Response to Anaerobic Soil Disinfestation. 2022 ASHS Annual Conference, Chicago, Illinois. HortScience S169. - Type: Conference Papers and Presentations Status: Awaiting Publication Year Published: 2022 Citation: W. Ouma, M. Dee, D. Butler, U. Shrestha, H. Rice, J. Littrell, B. Ownley. 2022. Control of Fusarium Root Rot in Strawberries through optimization of Anaerobic Soil Disinfestation with Biostimulants. 2022 American Phytopathology Society Annual Meeting, Plant Health, Pittsburgh, Pennsylvania. - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Butler, D.M. 2022. Anaerobic Soil Disinfestation. ASHS Annual Meeting Workshop, Doing More with Less: Reducing Fumigant Use with Alternative Production Systems. HortScience S34. ****Progress**** 09/01/20 to 08/31/21 ****Outputs**** Target Audience: The target audience during this second year of the project has largely been students and postdoctoral associates working on the project studies. We have also begun outreach to the scientific community through abstracts and scientific presentations, to grower, extension, and NRCS communities through outreach activities and presentations, and to undergraduate and graduate students through development of class lecture and lab activities. Changes/Problems: The impact of the COVID-19 pandemic and its effects on the ability to conduct timely, on-site work was less of a constraint compared to the first year of this project, but still hindered progress. We are closer to our targeted timeline than at the end of the first years reporting period, and will make significant efforts to meet our timeline in the coming reporting period. What opportunities for training and professional development has the project provided? Multiple undergraduate and graduate students, and a postdoctoral researcher participated in the project during the reporting period, providing numerous opportunities

for professional development and training in research methods related to soil disinfestation in organic strawberry systems. How have the results been disseminated to communities of interest? During the reporting period multiple activities were conducted to disseminate results to communities of interest. First, multiple scientific presentations (see abstracts in publications) were presented to disseminate results to the broader scientific community. Second, three workshops (a total of five presentations) were conducted to train farmers, extension personnel, and NRCS personnel on soil disinfestation systems. This included two presentations to UT Extensions New Farmer's Academy (a training program for early career farmers) at the field research site, two presentations to NRCS agents in Tennessee as part of their "Working with Organic Farmers" training series, and one presentation to southern region extension agents on soil amendment use in small fruit production systems as part of the annual training provided by the Southern Region Small Fruits Consortium. Lastly, prototype lab modules were developed and evaluated with undergraduate and graduate students at the University of Tennessee, who gained exposure to research methods and current understanding of soil disinfestation in organic horticultural systems. What do you plan to do during the next reporting period to accomplish the goals? In the coming reporting period, we plan to complete data analysis and manuscript preparation as part of Obj. 1, complete greenhouse/growth chamber studies associated with Obj. 2, implement the repeated field trial as part of Obj. 2, begin growth chamber and laboratory trials as part of Obj. 3, and complete development of educational materials associated with Obj. 4. We expect to continue outreach to the scientific community, grower groups, extension personnel, NRCS personnel, and undergraduate/graduate students through multiple venues. ****Impacts**** What was accomplished under these goals? The COVID-19 pandemic and its effects on the ability to conduct timely, on-site work remained a constraint into the second year of this project. However, we have largely completed data collection for Obj. 1, implemented field and growth chamber/greenhouse studies under Obj. 2, and developed and tested course laboratory activities under Obj. 4. In Obj. 1, we evaluated wheat cover crop residue as an ASD amendment with varying lignin to N ratio (L:N) on survival of an inoculated isolate of *Fusarium oxysporum* (Fo) causing root rot of strawberry and indigenous soil fungi following soil treatment by ASD. Treatments included 1) vegetative stage wheat residue, 1:1 L:N, 2) reproductive stage wheat residue, 2:1 L:N, and 3) mature wheat residue, 7:1 L:N, 4) a wheat bran control, and 5) a non-amended anaerobic control. The ASD experiment was conducted in pots with sandy soil in a growth chamber at 25-35°C, and repeated. All treated pots showed high anaerobic condition except for the mature wheat stage and the anaerobic control. Wheat residue with low L:N (1:1 and 2:1) substantially reduced recovered Fo inoculum populations compared to other treatments, with no negative impact on indigenous *Trichoderma* spp. or *Aspergillus* spp. Our results suggest that low L:N of wheat cover crop residue is needed to suppress the pathogenic Fo during ASD. We expect to complete data analysis and submit a manuscript for review in the next reporting period. In Obj. 2., greenhouse/growth chamber and field (high tunnel) studies were established at the end of the reporting period (late summer/early fall 2021). For Obj. 4, we developed two lab activity modules, evaluated with students, and will be editing based on student and instructor feedback in the next reporting period. ****Publications**** - Type: Conference Papers and Presentations Status: Published Year Published: 2020 Citation: Shrestha, U., B. H. Ownley, J. H. Rice, R. J. Vichich, and D. M. Butler*. 2020. Susceptibility of *Rhizoctonia solani* and *Fusarium oxysporum* to volatile fatty acids. Proceedings of the Annual International Research Conference on Methyl Bromide Alternatives, 8-1 to 8-3. - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Shrestha, U., J. Hollis Rice, J. Littrell, B.H. Ownley, D.M. Butler*. 2021. ASD amendment lignin to nitrogen ratio affects mortality of *Fusarium oxysporum* inoculum. American Phytopathology Society Annual Meeting, Plant Health 2021 (online). - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Shrestha, U.*, B.H. Ownley, D.M. Butler. 2021. Anaerobic/Biological Soil Disinfestation: Effects on Survival of Soilborne Pathogens and Beneficial Organisms. ASHS Annual Meeting Workshop, Strategies and Challenges for Implementing Anaerobic Soil Disinfestation. HortScience 56(9), S150.

PROGRESS

2019/09 TO 2020/08 Target Audience: The target audience during this first year of the project has largely been students and postdoctoral associates working on the initial project studies. Changes/Problems: The impact of the COVID-19 pandemic and its effects on the ability to conduct timely, on-site work were a major constraint to the first year of this project given that most proposed work at this stage could not be conducted remotely. We were able to make substantial progress on Obj. 1, and hope that we will be able to be closer to our targeted time line in the coming year. What opportunities for training and professional development has the project provided? Multiple undergraduate and graduate students, and a postdoctoral researcher participated in the project during the reporting period, providing numerous opportunities for professional development and training in research methods related to soil disinfestation in organic strawberry systems. How have the results been disseminated to communities of interest? Nothing Reported What do you plan to do during the next reporting period to accomplish the goals? In the coming reporting period, we plan to complete pot studies in Obj. 1 with an additional pathogen (*Pythium* spp.), and begin pot and field studies associated with Obj. 2. We expect that we will present our first

results at scientific meetings (American Phytopathological Society), begin Obj. 3 lab work, and begin development of educational materials associated with Obj. 4.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? The impact of the COVID-19 pandemic and its effects on the ability to conduct timely, on-site work were a major constraint to the first year of this project given that most proposed work at this stage could not be conducted remotely. We were able to make substantial progress on Obj. 1, and hope that we will be able to be closer to our targeted time line in the coming year. For Obj. 1, wheat cover crop residue lignin to N ratio at three stages was evaluated for optimizing anaerobic soil disinfestation (ASD) to reduce survival of *Fusarium oxysporum* (Fo). Fo isolates were collected strawberry in Tennessee. Inoculum was produced using presoaked organic oats autoclaved twice at 121°C for 15 min. The Fo isolate was grown on PDA and mycelial plugs (5-10mm) were transferred to autoclaved oats and incubated under fluorescent light for 15 days at room temperature. After gaining full colonization of oats, the Fo inoculum was dried for 24 hours and stored at 4°C. Packets with 2 g of inoculum were prepared using nylon bags for growth chamber pot studies. The growth chamber study used pots filled with 1.4 kg soil mixture (soil:sand=1:1) and wheat amendments at 3 lignin:N ratios. Temperatures were 25°C for 10 hours and 35°C for 14 hours to simulate a soil temperature regime in the southeastern U.S. during the time of a summer ASD treatment prior to fall strawberry planting. The treatment amendments included ground wheat residues at three wheat growth stages with a range of lignin:N ratios (wheat vegetative stage, 1:1 lignin:N ratio, reproductive stage, 2:1 ratio, and harvest stage, 7:1 ratio). A positive control with wheat bran as the carbon source was included. The total carbon rate for each treatment was maintained at 4 mg C / of soil. An anaerobic, non-amended & irrigated control and an aerobic, non-amended & irrigated control were also included. The Fo inoculum was introduced at 3-5 cm depth, iron oxyhydroxide coated (IRIS) tubes and manganese oxide (MnO) painted stakes were installed at 10 cm depth following amendment incorporation to assess soil anaerobic conditions. Pots were irrigated to saturation, covered with black polyethylene mulch, and incubated in the growth chamber. Pots were arranged in a completely randomized design. Following ASD treatment, IRIS tubes, MnO stakes were removed and percentage of Fe or Mn paint removal by soil reduction was assessed. Fo packets were removed and propagule survival were assessed by serial dilution plating of recovered inoculum onto Nash-Snyder selective media. Soil samples were collected and extracted to evaluate soil concentrations of volatile fatty acids (VFAs). Preliminary results showed high anaerobic condition generated in the wheat treatments with low lignin to N ratios (1 and 2:1), that was comparable with wheat bran treatment (41 to 57% Mn; 35 to 45% Fe paint removal). The high lignin to N ratio (7:1; ~20% paint removal) indicated low anaerobic conditions and was not different from anaerobic control (0% Mn; 13% Fe paint removal). Mortality of Fo inoculum was highest in the low wheat lignin:N ratio (1:1) amended pots compared to all other treatments. When compared to the anaerobic control, wheat at low lignin:N reduced Fo population by 98%. **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 No publications reported this period. ** **

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Research and Outreach to Support Transitioning to Organic Cotton Production in Central and Gulf Coast Texas

Accession No.	1020564
Project No.	TEX09803
Agency	NIFA TEX\
Project Type	OTHER GRANTS
Project Status	NEW
Contract / Grant No.	2019-51106-30192
Proposal No.	2019-03508
Start Date	01 SEP 2019
Term Date	31 AUG 2022
Grant Amount	\$499,731
Grant Year	2019
Investigator(s)	Bagavathiannan, M.
Performing Institution	TEXAS A&M UNIVERSITY, 750 AGRONOMY RD STE 2701, COLLEGE STATION, TEXAS 77843-0001

NON-TECHNICAL SUMMARY

Cotton is an important crop in the United States (US) and Texas is the leading producer of this commodity, planted on about 7.8 million acres in 2018 (NASS 2019), with a direct sales value of \$2.7 billion and an economic impact of \$24 billion annually inclusive of all products and services (OTA 2019). In addition to the fiber, cottonseed is an important component of the industry, which is often overlooked. In 2018, Texas produced about 2 million tons of cottonseed, with a sales value of \$341 million (NASS 2019). Though the majority (~97%) of the current US organic cotton is produced in Texas, the acreage is still very low (approx. 14,000 acres, only 0.2% of all cotton acres in TX) and is mainly concentrated in the Texas High Plains. There is a high potential for expanding organic cotton production in the state, particularly in the Central and Gulf Coast Texas regions, but knowledge gaps exist for transitioning from conventional to organic cotton, including organic defoliation methods, conservation tillage, and cover crop systems for weed management, soil nutrient dynamics and greenhouse gas mitigation potential. The overarching goal of this project is to help bridge the knowledge gaps through interdisciplinary research and outreach. Research: Developing organically-approved defoliation methods can greatly benefit organic cotton growers. In this regard, some of the nonsynthetic natural product herbicides developed for use in other organic systems can be evaluated for their utility as a defoliant in cotton. OMRI ensures that the constituents of the products meet the requirements of the NOP guidelines, but it does not evaluate the efficacy of the products for their herbicidal properties. Different concentrations and combinations of plant essential oils and other natural products will be tested for their effectiveness as a defoliant for cotton. Weeds present the most important challenge for organic crop production, and economical weed management is touted as the prime constraint for transitioning to organic agriculture (Cavigelli et al. 2008; Posner et al. 2008; Liebman and Davis 2009). Removal of tillage from organic production systems warrants the development of robust non-chemical weed management tactics. Integration of cover crops can provide effective weed management, in addition to improving soil health and providing other ecosystem benefits (Teasdale 1996; Gallandt et al. 1999; Hartwig and Ammon 2002). Growers can take advantage of the extended growing season (Sep to Nov) in the Central and Gulf Coast regions and plant a short-duration cover crop after cotton harvest (typically harvested in the region by the end of Aug) to suppress post-harvest recruits of problematic summer-annual weeds such as Palmer amaranth (*Amaranthus palmeri*), waterhemp (*A. tuberculatus*), and ragweed parthenium (*Parthenium hysterophorus*) through live biomass. Further, late fall-planted winter cover crops can facilitate in-season suppression of winter annual weeds through live biomass and suppression of summer annual

weeds through cover crop residues. Agronomic practices in Central and Gulf Coast Texas are currently dominated by intensive tillage systems (i.e. conventional tillage). Many organic farmers rely on tillage and row cultivation for weed management (Teasdale and Mirsky 2015). This poses a great challenge to organic farming. There is unequivocal research evidence that tillage and repeated cultivations degrade soil quality (Wienhold and Halvorson 1998; Lal 2015). Long-term continuous cultivation also leads to soil erosion, increased emission of GHGs such as CO₂, and reduced carbon sequestration, in addition to added labor and energy costs. In this research, we will evaluate various summer as well as winter cover crop species and compare conventional and conservation tillage systems. Observations will include soil moisture dynamics, weed suppression, soil fertility, greenhouse gas emissions, and crop biomass and yield. Outreach and education Developing and delivering pertinent outreach activities is imperative to provide critical production information and best management practices to growers in a timely manner. Demonstration of the benefits of these best production practices on yield and economics will particularly entice other farmers considering on transitioning to organic production. To be effective, these demonstrations should be conducted in participating grower fields. This will allow the farmers to see for themselves the benefits of improved production practices, and the information will subsequently disseminate to other farmers through 'word of mouth'. Further, providing training and support on organic certification process will assist growers and offer a positive experience while transitioning to organic cotton production. There is a lack of educational material and outreach bulletins on organic cotton production. Developing robust educational material on different aspects of organic production will allow the growers develop a good understanding of the basics of organic production. Engagement of County Extension agents should be an important component of the outreach plan because they are the first point of contact for information by the growers. Additionally, training graduate and undergraduate students with organic production practices can provide long-term benefits since these students are the ones who will greatly influence the future of organic production, as farmers, rented operators, field agronomists, research scientists, extension personnel, policy makers, as well as progressive members of the general public. Our outreach and education plan will address these critical aspects.

OBJECTIVES

The long-term goal of the proposed project is the expansion of organic cotton production in Texas. Although organic cotton fetches a high price premium and is growing in demand, very few farmers were transitioning to these systems due to some key barriers. We believe that these barriers can be addressed through pertinent research and outreach activities, and providing direct support to growers interested in transitioning to organic cotton production. Further, we believe that transitioning to organic cotton requires research knowledge developed based on the interrelationships of basic biophysical processes, and the impact of economic and market forces. Such research must include both basic and applied aspects with full partnership and engagement of stakeholder groups, which is addressed in this study. The following project objectives were determined based on the assessments we made from interacting with the stakeholders. 1. Conduct research and on-farm demonstrations on defoliation methods to fill a critical knowledge gap (that support insurance programs) for organic cotton production in Central and Gulf Coast Texas regions (Research Objective) 2. Conduct additional agronomic research and on-farm demonstrations on the influence of cover cropping and conservation tillage practices on weed management, water-yield relations, soil nutrient dynamics and health, and greenhouse gas emissions, and develop best management practices that optimize economic returns and ecosystem services in organic cotton production (Research Objective) 3. Develop and deliver an educational and outreach program for efficient transfer of project results to various stakeholders to facilitate transitioning to organic cotton production in Central and Gulf Coast Texas regions (Education and Outreach Objective)

APPROACH

1. Research locations. The rigorous field data collection for the objectives 1 and 2 will be carried out in a transitioning organic site at the Texas A&M field research facility near College Station, TX. A field demonstration site will be established at the participating farmer (Zack Yanta)'s transitioning organic cotton field in Lavaca County, TX to disseminate the findings to broader stakeholder groups. 2. Research Methodology and data collection 2.1. Objective 1 Defoliant evaluation at College Station Field experiments will be conducted in College Station, TX over a two-year period (summer 2019, 2020) to evaluate various nonsynthetic products that meet NOP standards for use as a cotton defoliant. This experiment will be conducted in a randomized complete block design with four replications. Each plot will consist of 4 rows (3 m) x 6 m. A total of 16 different treatments will be evaluated: acetic acid (vinegar) at 20 and 30% concentrations, citric acid, neem oil, castor oil, clove oil, clove oil + citric acid, cinnamon oil, thyme oil, acetic acid 23% + citric acid 14% (Alldown®), citric acid 5% + garlic 0.2% (Alldown® Green), clove oil 45.6% (Matran® 2), d-limonene (Avenger®), thyme 10.4% + clove oil 10.1%

(Xpress®), and ammonium nanoate (Axxe®). A non-sprayed control will also be maintained for comparison. 2.2. Objective 2 Conservation tillage and cover crop experiment at College Station A three-year (2019-2022) transitional organic experiment will be established at the Texas A&M field research facility near College Station, TX. The experiment will be arranged in a split-plot design with four replications. The main-plot factor is tillage system, with 2 levels: strip-tillage (a conservation tillage practice) and conventional tillage. The sub-plot factor is 10 levels: 4 fall-planted covers (cereal rye, haygrazer oats, Austrian winter peas, and a mix of all three species), with and without a late-summer planted cowpea double cover cropping. These cover crop choices are also ideal due to a lack of weediness potential. Additionally, a weedy fallow and a weed-free bareground check treatment will be included for comparison. Each sub-plot will be 12 rows (9 m) wide x 15 m long. Field Data collection (i) Weed population dynamics: We will estimate weed seedbank dynamics, seedling emergence pattern and weed control in each plot. Weed biomass will be estimated from six 0.5 m² random quadrats within each plot at the end of the growing season. (ii) Soil moisture - impact of cover crops: Soil moisture measurements will be carried out at daily intervals using automatic moisture sensors (Stevens® GroPoint Profile) at three different depth profiles, 0-15 cm, 15-30 cm, and 30-45 cm. Water use efficiency estimates will be derived using crop biomass and yield data. (iii) Crop growth and yield: The physiological and growth parameters for cotton and cover crops will be measured at mid-season and at crop maturity. Specifically, observations will be carried out on plant height, leaf area index (LAI), light interception (ACCUPAR LP-80 ceptometer), SPAD value (SPAD 502 Plus Chlorophyll Meter), biomass production, and yield. (iv) Soil physical properties: Soil physical properties such as bulk density, water infiltration rate, and aggregate stability are important indicators of soil health. Detailed measurements of soil physical properties will be taken following cotton harvest in the first year and third year of experiments. (v) Soil fertility: Soil samples for conventional nutrient testing (0-15 cm) and for residual NO₃-N (0-15 cm, 15-45 cm) will be taken as composites for each plot and analyzed at the Texas A&M University Soil Testing Laboratory. Tissue testing for nutrients (N, P, K, S, Ca, Mg, and micronutrients) will be performed midseason on each plot. (vi) Nutrient cycling: Soil nitrogen (N), carbon (C), and phosphorus (P) as totals will be measured for each plot prior to cotton planting. Crop and cover crop residue (as appropriate for the treatment) will be measured for total N, C, and P. Root density will be estimated as percent inoculated roots observed out of ten randomly selected root segments from each plot. (vii) GHG measurements: GHG measurements will be made at weekly intervals using static chambers. Dr. Rajan has static chambers available for use in this project. Gas samples will be drawn from the chamber headspace using gas-tight syringes at 0, 10, 20 and 30 minute intervals for flux calculation. Gas samples will be analyzed for CO₂, N₂O and CH₄ concentrations using a gas chromatograph equipped with an electron capture detector. To assess all three GHGs together, N₂O and CH₄ emissions will be expressed in terms of CO₂-carbon equivalents (for example, N₂O is multiplied by 298 and CH₄ is multiplied by 34). (viii) Indicators of soil biological activity and health: In this study, we will measure microbial biomass carbon and nitrogen. We will use the chloroform fumigation and extraction method for determining soil microbial biomass carbon and nitrogen (Vance et al. 1987). Chloroform fumigated and non-fumigated samples will be extracted using K₂SO₄ and samples will be analyzed for total organic carbon and total nitrogen at Dr. Rajan's laboratory using an Elementar vario MAX cube CN analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Additionally, soil respiration measurements will be made using static chambers and will be collected as part of the GHG measurements described above. Economic analysis The data from experimental and demonstration fields will be used to build enterprise budgets to understand the associated costs, revenues, and enterprise profits. This will serve as a representative model for farmers and allow them to estimate the net revenue in their farms associated with different improved management practices. The price volatility (historical/implied) will be used to analyze the economic risk. The field measurements will be used to conduct a scenario analysis with a hypothetical cap-and-trade mitigation policy. 2.3. Objective 3 Outreach and educational plan: Guided by the research findings, we will develop appropriate educational materials in Years 2 and 3, to meet the needs of Texas growers interested in transitioning to organic cotton production. i) Field days: Stakeholder field days will be conducted in June and October of project Years 2 and 3, utilizing the demonstration plots to be established at Mr. Yanta's transitioning organic cotton farm in Lavaca County. Participants will have an opportunity to witness the benefits of best management practices for organic cotton production. Further, this event will facilitate experience sharing among the stakeholders. ii) Training through Texas A&M AgriLife Extension: Dr. McGinty will organize training sessions and conduct pertinent outreach activities in the focus region during the Years 2 and 3 of the project. The proposed activities include, but not limited to: Develop Organic System Plan training modules, both Trainer and Grower versions, with topics including the certification process, plan development, crop establishment, weed management, soil and fertility management, insect pest/disease management, organic integrity, and harvest/handling; b) Provide training workshops for County Extension Agents (CEAs); c) Enable CEAs to provide OSP training to growers, with options for in-person and remote participation; d) Provide training workshops for growers; e) Organize field day events to demonstrate and discuss successful organic best management practices; f) Develop extension bulletins to disseminate research findings; and g) Routinely discuss with organic cotton producers about what practices have worked and what have not. All educational materials will be made available through the Texas A&M AgriLife Extension website (<<https://agrilifeextension.tamu.edu>>).

Progress 09/01/22 to 08/31/23 Outputs (N/A) Impacts What was accomplished under these goals? Objective 1: Conduct research on defoliation methods for organic cotton Field experiments were repeated in 2022 to test different non-synthetic herbicides for their effectiveness as cotton defoliants. Both leaf injury and leaf drop were evaluated at 7 and 14 days after application. The study provided vital information on the potential use of select non-synthetic products as defoliants. The manuscript is ready for submission to Weed Technology journal. Objective 2: Influence of cover cropping and conservation tillage practices on weed management, water-yield relations, soil nutrient dynamics and health, and greenhouse gas emissions The field experiments were concluded during the 2022 field season. An additional soil incubation study was conducted under laboratory conditions during 2022-23. The static chamber technique was used to monitor weekly emissions of carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄). Due to poor crop performance in strip-till plots, measurements were taken only on conventional tillage plots. Greenhouse gas sampling was carried out at weekly intervals until cotton harvest. Twelve ml pre-evacuated vials were used to collect gas samples at 00, 10, 20- and 30-minute intervals after closing the chamber to capture the gas flux. The samples were analyzed at Texas A&M AgriLife Center-Amarillo each week. Soil moisture and temperature were monitored at 30-minute intervals throughout the cotton growth period using CS655 sensors connected to CR-1000 dataloggers (Campbell Scientific). The greenhouse gas chambers and soil moisture sensors were reinstalled again after cover crop planting in the fall of 2021 and continued to monitor the emissions and soil moisture dynamics during the cover crop and cotton growing period of 2021-2022. Multiple soil incubation studies were conducted from 2020 to 2023 with soils collected from the organic field to assess the mineralization dynamics and associated greenhouse gas emissions of cover crop residues with poultry manure and biochar. The emissions varied by year, while cover crops showed promising results in reducing N₂O emissions and helped conserve soil moisture. Objective 3: Education and outreach Outreach was conducted at 10 county extension meetings during the summer of 2023, with a total attendance of about 400. These events reached producers in San Patricio, Nueces, Jim Wells, Kleberg, Jackson, Victoria, Calhoun, Fayette, and Colorado counties. Additionally, findings were presented at the Texas Plant Protection Association Meeting, which is widely attended by growers and crop consultants. Publications Type: Theses/Dissertations Status: Published Year Published: 2023 Citation: Salehin M (2023) Impact of organic management practices on soil greenhouse gas emissions from cotton-winter cover crop systems in east-central Texas. PhD Dissertation, Department of Soil and Crop Sciences, Texas A&M University Type: Journal Articles Status: Under Review Year Published: 2024 Citation: Salehin SMU, Rajan N, Casey KD, Mowrer JE, Somenahally A, Bagavathiannan M (2024). Greenhouse gas emissions during decomposition of cover crops and manure with simulated tillage in long-term soil incubations. Soil Science Society of America Journal (Manuscript under review). Type: Journal Articles Status: Other Year Published: 2024 Citation: Maity A, Hathcoat D, Bagavathiannan M (2024) Evaluation of organic herbicides as defoliants in cotton production. Weed Technology (under communication) Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Salehin, S. M. U., Rajan, N., Poudyal, C., & Bagavathiannan, M. V. (2023) Estimation of Cover Crop Biomass Using UAV-Mounted Multispectral Sensors \Abstract\ ASA, CSSA, SSSA International Annual Meeting, St. Louis, MO. Type: Conference Papers and Presentations Status: Published Year Published: 2023 Citation: Salehin, S. M. U., Rajan, N., Mowrer, J. E., Casey, K. D., Somenahally, A., & Bagavathiannan, M. V. (2023) Reducing Soil Greenhouse Gas Emissions with Biochar during Cover Crops and Manure Decomposition \Abstract\ ASA, CSSA, SSSA International Annual Meeting, St. Louis, MO. Progress 09/01/19 to 08/31/23 Outputs Target Audience: The key target audiences reached during this reporting period include organic farmers, conventional farmers, county extension agents, Texas Organic Farmers and Gardeners Association (TOFGA), and Cotton Incorporated. Changes/Problems: The project was significantly impacted by COVID-19 related disruption of activities during 2020 and 2021. In 2022, a severe drought and extremely hot weather conditions in TX during the majority of the summer affected some of the field activities. However, we were able to simulate greenhouse gas emissions utilizing laboratory incubation experiments as well, which provided valuable insights into the influence of cover crop residue retention on the emission dynamics. What opportunities for training and professional development has the project provided? The project has provided training on field agronomy and organic weed management methods for an MS student, training on soil nutrient and microbial diversity analysis for another MS student, and training on greenhouse gas measurements and analysis for a PhD student. This project also provided opportunities for two undergraduate students for conducting short research projects. Additionally, seven undergraduate student workers were trained on various field and lab activities. How have the results been disseminated to communities of interest? Results were communicated to the broader scientific community through presentations at various scientific meetings, including the ASA-CSSA-SSSA annual meeting, Southern American Society of Agronomy Meeting, Weed Science Society of America Annual Meeting, Beltwide Cotton Conference, and the Southern Weed Science Society Annual Meeting. Informal discussions were made with Cotton Incorporated and the Texas Organic Farmers and Gardeners Organization (TOFGA) on salient research findings. What do you plan to do during the next reporting period to accomplish the goals? Nothing Reported Impacts What was accomplished under these goals? Objective 1: Conduct research on defoliation methods for organic cotton Field experiments were repeated in 2022 to test different non-synthetic

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J., Somenahally, A., Mowrer, J. E., & Bagavathiannan, M. V., 2021. Emission and Sequestration of Greenhouse Gases in Organically Managed Cotton Cropping Systems \Abstract\. ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT. Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Salehin, S.M.U., Rajan, N., Casey, K., Mowrer, J., and Bagavathiannan, M., 2021. Effects of Different Cover Crops and Tillage on Greenhouse Gas Emissions in Soil Incubation. \Abstract\. In ASA Southern Regional Branch Annual Meeting, Virtual-2021. American Society of Agronomy. Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Barth, M., Salehin, S. M. U., Mowrer, J. E., Rajan, N., & Bagavathiannan, M., 2021. An Agronomic Evaluation of Tillage and Cover Crops in Organic Cotton Production \Abstract\. ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT. 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In: Proceedings of Southern American Society of Agronomy Annual Meeting (Virtual) Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Us-Salehin M, Rajan N, Casey K, Mowrer J, Tomlinson P, Bagavathiannan M (2021) Effects of cover crops on greenhouse gas emissions, soil temperature, and soil moisture dynamics in organic cotton. In: Proceedings of the ASA-CSSA-SSSA Annual Meeting (Virtual) Type: Conference Papers and Presentations Status: Published Year Published: 2020 Citation: Salehin, M.U., Rajan, N., Casey, K., Mowrer, J., Tomlinson, P., Bagavathiannan, M. 2020. Effects of cover crops on greenhouse gas emissions, soil temperature, and soil moisture dynamics in organic cotton. 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Informal discussions were made with Cotton Incorporated and the Texas Organic Farmers and Gardeners Organization (TOFGA) on salient research findings. What do you plan to do during the next reporting period to accomplish the goals? We have obtained a no-cost extension FY2022-2023 to complete pending activities. A significant effort will be dedicated to data analysis and manuscript writing. ****Impacts**** What was accomplished under these goals? Objective 1: Conduct research on defoliation methods for organic cotton Field experiments were repeated in 2022 to test different non-synthetic herbicides for their effectiveness as cotton defoliant. Both leaf injury and leaf drop were evaluated at 7 and 14 days after application. The study provided vital information on potential use of select non-synthetic products as defoliant. Data analysis is complete and a journal manuscript is currently being developed. Objective 2: Influence of cover cropping and conservation tillage practices on weed management, water-yield relations, soil nutrient dynamics and health, and greenhouse gas emissions Cover crops were planted in late fall

2021, following the harvest of cotton. The treatments included oats, Austrian winter pea, purpletop turnip and a mix of all three covers. Cover crops were terminated using a roller crimper, and the cotton crop was planted following a conventional till or strip-till. Cotton was planted and maintained organically, and the trial was used for greenhouse gas measurements and soil nutrient dynamics assessments. Greenhouse gas measurements: The static chamber technique was used to monitor weekly emissions of carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄). Due to poor crop performance in strip-till plots, measurements were taken only on conventional tillage plots. Greenhouse gas sampling was carried out at weekly intervals until cotton harvest. Twelve ml pre-evacuated vials were used to collect gas samples at 00, 10, 20- and 30-minute intervals after closing the chamber to capture the gas flux. The samples were analyzed at Texas A&M AgriLife Center-Amarillo each week. Soil moisture and temperature were monitored at 30-minute intervals throughout the cotton growth period using CS655 sensors connected to CR-1000 dataloggers (Campbell Scientific). The greenhouse gas chambers and soil moisture sensors were reinstalled again after cover crop planting in the fall of 2021 and continued to monitor the emissions and soil moisture dynamics during the cover crop and cotton growing period of 2021-2022. A soil incubation study was done in 2022 with soils collected from the organic field to assess the mineralization dynamics and associated greenhouse gas emissions of cover crop residues with poultry manure and biochar. The emissions varied by year, while cover crops showed promising results in reducing N₂O emissions and helped conserve soil moisture. Soil nutrient dynamics and microbial activity: Soil samples for fertility/plant available nutrients were collected in 2021-22. Samples were taken for the 0-15 cm and 15- 38 cm soil depths. Soil samples for controlled incubations were collected in spring 2022. These samples were used to assess microbial activity in the top 10-cm profile. Incubations were conducted to assess general heterotroph activity (as CO₂ respiration), carbon, nitrogen, and phosphorus mineralization as a function of the imposed treatments. Results show that up to 90% of the carbon added to the soil from cover crop biomass residue can be mineralized to CO₂ within two months during the summer in Central Texas. Brassicas tend to exhibit narrow C:N ratios similar to legumes and, therefore, break down faster than grass/grain covers and can also lead to more N₂O emissions than grass/grain covers. Data analysis is currently ongoing. Objective 3: Education and outreach Outreach was conducted at 11 county extension meetings during the summer of 2022, with a total attendance of 440. These events reached producers in San Patricio, Nueces, Jim Wells, Kleberg, Jackson, Victoria, Calhoun, Fayette, and Colorado counties. Additionally, findings were presented at the Texas Plant Protection Association Meeting which is widely attended by growers and crop consultants. The carbon mineralization data has been communicated at 5 farmer meetings on the subjects of soil carbon market and cover crop benefits. A factsheet was produced on organic methods for weed control in cotton production.

****Publications**** - Type: Theses/Dissertations Status: Published Year Published: 2022 Citation: Barth M (2022) Evaluating reduced tillage, cover crops, and living mulches for weed management in cotton. MS Thesis, Department of Soil and Crop Sciences, Texas A&M University - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Salehin, S. M. U., Rajan, N., Murphree, P., Mowrer, J. E., Casey, K. D., & Bagavathiannan, M. V., 2021. Greenhouse Gas Emissions during Long-Term Incubation of Organically Managed Soils with Different Cover Crops. \Abstract\ ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT. - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Salehin, S. M. U., Rajan, N., Miyataka, N., Casey, K. D., Tomlinson, P. J., Somenahally, A., Mowrer, J. E., & Bagavathiannan, M. V., 2021. Emission and Sequestration of Greenhouse Gases in Organically Managed Cotton Cropping Systems \Abstract\ ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT. - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Salehin, S.M.U., Rajan, N., Casey, K., Mowrer, J., and Bagavathiannan, M., 2021. Effects of Different Cover Crops and Tillage on Greenhouse Gas Emissions in Soil Incubation. \Abstract\ In ASA Southern Regional Branch Annual Meeting, Virtual-2021. American Society of Agronomy. - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Barth, M., Salehin, S. M. U., Mowrer, J. E., Rajan, N., & Bagavathiannan, M., 2021. An Agronomic Evaluation of Tillage and Cover Crops in Organic Cotton Production \Abstract\ ASA, CSSA, SSSA International Annual Meeting, Salt Lake City, UT. - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Murphee P, Mowrer J (2022) Tillage practice affects soil CO₂ respiration more than cover crops the second year of organic cotton study. Soil Survey and Land Resource Workshop, College Station, TX - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Murphee P, Mowrer J (2022) Soil carbon and nitrogen mineralization are affected by tillage and cover cropping in an organic cotton system in Texas. American Society of Agronomy Southern Branch, New Orleans, LA. - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Barth M, Salehin M, Mowrer J, Rajan N, Bagavathiannan M (2021) An agronomic evaluation of tillage and cover crops in organic cotton production. In: Proceedings of the 2021 American Society of Agronomy Annual Meeting, Salt Lake City, UT - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Barth M, Bagavathiannan M (2021) Evaluation of living mulch species and their effect on weed pressure in cotton. In: Proceedings of the 2021 American Society of Agronomy Annual Meeting, Salt Lake City, UT - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Salehin, S.

M. U., Rajan, N., Casey, K. D., Tomlinson, P. J., Miyanaka, N., Mowrer, J. E., & Bagavathiannan, M. V., 2022. Greenhouse Gas Emissions and Sequestration in Organic Cotton Systems with Different Cover Crops. \Abstract\ ASA, CSSA, SSSA International Annual Meeting, Baltimore, MD. - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Salehin, S. M. U., Rajan, N., Casey, K. D., Mowrer, J. E., Somenahally, A., & Bagavathiannan, M., 2022. Biochar Reduces Greenhouse Gas Emissions during Decomposition of Cover Crops and Manures: Results from Soil Incubation \Abstract\ ASA, CSSA, SSSA International Annual Meeting, Baltimore, MD. - Type: Conference Papers and Presentations Status: Published Year Published: 2022 Citation: Salehin, S. M. U., Barth, M., Rajan, N., Bagavathiannan, M., & Mowrer, J. E., 2022. Prospects and Challenges of Organic Cotton Production in East-Central Texas. \Abstract\ ASA, CSSA, SSSA International Annual Meeting, Baltimore, MD. ****Progress**** 09/01/20 to 08/31/21 ****Outputs**** Target Audience: The key target audiences reached during this reporting period include organic farmers, conventional farmers, county extension agents, Texas Organic Farmers and Gardeners Association (TOFGA), and Cotton Incorporated. Changes/Problems: The project was significantly impacted by COVID-19 related disruption of activities. Further, continuous rain events for more than 6 weeks during early summer 2021 led to severe weed interference and complete crop failure. We were able to replant cotton, but was a late planting timing for the region, which pushed the harvest timing as well. Also, weed pressure was very severe in the strip-till plots and the organic methods of weed control we implemented were not sufficient in these plots due to larger than optimum weed sizes. This situation led to poor cotton growth and yield in the strip-till plots. Greenhouse gas emission measurements were not conducted in the strip-till plots due to severe weed growth and poor cotton performance. It is our opinion that inter-row cultivation is critical to ensure successful cotton production in the strip-till plots, until other effective and selective control methods are developed. The learnings from this year will be helpful for better addressing these challenges next year. What opportunities for training and professional development has the project provided? The project has provided training on field agronomy and organic weed management methods for an MS student, training on soil nutrient and microbial diversity analysis for another MS student, and training on greenhouse gas measurements and analysis for a PhD student. This project also provided opportunities for two undergraduate students for conducting short research projects. Additionally, four undergraduate student workers were trained on various field and lab activities. How have the results been disseminated to communities of interest? Results were communicated to the broader scientific community through presentations at various scientific meetings, including the ASA-CSSA-SSSA annual meeting, Southern American Society of Agronomy Meeting, Weed Science Society of America Annual Meeting, Beltwide Cotton Conference, and the Southern Weed Science Society Annual Meeting. Informal discussions were made with Cotton Incorporated and the Texas Organic Farmers and Gardeners Organization (TOFGA) on salient research findings. What do you plan to do during the next reporting period to accomplish the goals? We will continue to make progress on all project objectives, building on the learnings from this past year. ****Impacts**** What was accomplished under these goals? Objective 1: Conduct research on defoliation methods for organic cotton Field experiments were conducted in summer 2020 to test different non-synthetic herbicides for their effectiveness as cotton defoliant. Both leaf injury and leaf drop were evaluated at 7 and 14 days after application. The study provided vital information on potential use of select non-synthetic products as defoliant. The experiment will be repeated during summer 2021. Objective 2: Influence of cover cropping and conservation tillage practices on weed management, water-yield relations, soil nutrient dynamics and health, and greenhouse gas emissions Cover crops were planted in late fall 2020, following the harvest of cotton. The treatments included oats, Austrian winter pea, purpletop turnip and a mix of all three covers. Cover crops were terminated using a roller crimper and the cotton crop was planted following a conventional till or strip-till. Due to prolonged rains during early summer 2021, weed control attempts have failed and weeds grew out of control. As a result, we had to terminate the trial and replant cotton, which was a late planting timing for our location. Routine field observations and measurements were carried out as required. Weed-related measurements: Soil seedbank samples were collected prior to planting cotton (first planting) during early summer 2021. The seedbank size was estimated by the grow-out method in the greenhouse. Weed seedling emergence was counted throughout the season in permanent quadrats marked in each plot. Weed biomass was measured in each plot prior to cotton harvest. Greenhouse gas measurements: The static chamber technique was used to monitor weekly emissions of carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄). Due to poor crop performance in strip-till plots, measurements were taken only on conventional tillage plots. Greenhouse gas sampling was carried out at weekly intervals until cotton harvest. Twenty ml pre-evacuated vials were used to collect gas samples at 00, 10, 20- and 30-minute intervals after closing the chamber to capture the gas flux. The samples were analyzed at Texas A&M AgriLife Center-Amarillo each week. Soil moisture and temperature were monitored at 30-minute interval throughout the cotton growth period using CS655 sensors connected to CR-1000 dataloggers (Campbell Scientific). The greenhouse gas chambers and soil moisture sensors will be reinstalled again after cover crop planting in fall 2021 to monitor the emissions and moisture dynamics during the cover cropping period. Soil nutrient dynamics and microbial activity: Soil samples for fertility/plant available nutrients were collected twice since fall 2020. Samples were taken for the 0-15 cm and 15- 38 cm soil depths. Soil samples for controlled

incubations were collected once, in spring 2021. These samples were used to assess microbial activity in the top 10 cm profile. Incubations are currently underway to assess general heterotroph activity (as CO₂ respiration), carbon, nitrogen, and phosphorus mineralization as a function of the imposed treatments. The results of the analyses from these samples will be correlated against cotton yield and tissue nutrient concentrations (plant uptake). Objective 3: Education and outreach Outreach was conducted at 9 county extension meetings in June, with a total attendance of 347. These events reached producers in San Patricio, Nueces, Jim Wells, Kleberg, Jackson, Victoria, Calhoun, Fayette, and Colorado counties. Additionally, findings were presented at the Texas Plant Protection Association Meeting which is widely attended by growers and crop consultants. More outreach activities are planned for next year. ****Publications**** - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Barth M, Bagavathiannan M (2021) Cover crops and tillage regime alter weed pressure in organic cotton production. In: Proceedings of the 2021 Southern Weed Science Society Annual Meeting (Virtual) - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Barth M, Bagavathiannan M (2021) Impact of tillage regime and cover cropping on weed dynamics in organic cotton production. In: Proceedings of the 2021 Weed Science Society of America Annual Meeting (Virtual) - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Us-Salehin M, Rajan N, Casey K, Mowrer J, Tomlinson P, Bagavathiannan M (2021) Greenhouse gas emissions, soil moisture and temperature dynamics with different cover crops in organic cotton. In: Proceedings of the 2021 Beltwide Cotton Annual Meeting (Virtual) - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Us-Salehin M, Rajan N, Mowrer J, Casey K, Bagavathiannan M (2021) Greenhouse gas emissions with different tillage and cover crops in organic cotton. In: Proceedings of Southern American Society of Agronomy Annual Meeting (Virtual) - Type: Conference Papers and Presentations Status: Published Year Published: 2021 Citation: Us-Salehin M, Rajan N, Casey K, Mowrer J, Tomlinson P, Bagavathiannan M (2021) Effects of cover crops on greenhouse gas emissions, soil temperature, and soil moisture dynamics in organic cotton. In: Proceedings of the ASA-CSSA-SSSA Annual Meeting (Virtual)

PROGRESS

2019/09 TO 2020/08 Target Audience: The key target audiences reached during this reporting period include organic farmers, conventional farmers, county extension agents, Texas Organic Farmers and Gardeners Association (TOFGA), and Cotton Incorporated. Changes/Problems: The project was significantly impacted by COVID-19 related disruption of activities. Cotton planting was delayed in the spring which led to high weed issues. Also, weed pressure was very severe in the strip-till plots and the organic methods of weed control we implemented were not sufficient in these plots due to larger than optimum weed sizes. This situation led to poor cotton growth and yield in the strip-till plots. Greenhouse gas emission measurements were terminated in the strip-till plots by mid-summer due to severe weed interference. Based on our experience, we propose to make a modification to our strip-till treatment. Specifically, we would like to change it to a reduced tillage system that includes strip tillage prior to cotton planting, followed by an inter-row cultivation during early cotton growth period to control weeds. It is our opinion that the inter-row cultivation is critical to ensure successful cotton production in the strip-till plots, until other effective and selective control methods are developed. What opportunities for training and professional development has the project provided? The project has provided training on field agronomy and organic weed management methods for an MS student, training on soil nutrient and microbial diversity analysis for another MS student, and training on greenhouse gas measurements and analysis for a PhD student. This project also provided opportunities for two undergraduate students for conducting short research projects. Additionally, four undergraduate student workers were trained on various field and lab activities. How have the results been disseminated to communities of interest? Results were communicated to the broader scientific community through presentations at the ASA-CSSA-SSSA annual meeting. Presentations are also planned for the upcoming Weed Science Society of America Annual Meeting. Informal discussions were made with Cotton Incorporated and the Texas Organic Farmers and Gardeners Organization (TOFGA) on salient research findings. What do you plan to do during the next reporting period to accomplish the goals? We will continue to make progress on all project objectives, building on the momentum generated this past year.

IMPACT

2019/09 TO 2020/08 What was accomplished under these goals? Objective 1: Conduct research on defoliation methods for organic cotton Progress: A preliminary test was conducted during fall 2019 with different organic herbicides for their effectiveness in desiccating/defoliating organic cotton. Based on the preliminary results, a detailed experiment was designed and conducted during summer 2020. A total of 23 treatments were tested. Data are currently being organized and analyzed. The experiment will be repeated during summer 2021. Objective 2: Influence of cover cropping and conservation tillage practices on weed management, water-yield

relations, soil nutrient dynamics and health, and greenhouse gas emissions Progress: The field study was established during fall 2019, with the planting of the cover crops. Cover crops were terminated using a roller crimper and the cotton crop was planted following a conventional till or strip-till. Routine field observations and measurements were carried out as required. Weed-related measurements: Soil seedbank samples were collected prior to planting cotton in spring and again after cotton harvest in fall. The seedbank size was estimated by the grow-out method in the greenhouse. Weed seedling emergence was counted throughout the season in permanent quadrats marked in each plot. Weed biomass was measured in each plot prior to cotton harvest. Greenhouse gas measurements: The static chamber technique was used to monitor weekly emissions of carbon dioxide (CO₂), nitrous oxide (N₂O) and methane (CH₄). Due to very low stand count in strip-till plots, measurements were taken only on conventional tillage plots for all four reps. First gas sampling was done in July followed by one sampling each week until cotton harvest. Twenty ml pre-evacuated vials were used to collect the gas samples and at 00, 10, 20- and 30-minute intervals after closing the chamber to capture the gas flux. The samples were sent to Dr. Kenneth Casey in Texas A&M AgriLife Extension Service at Amarillo for analyzing the samples in gas chromatographs each week. Soil moisture and temperature dynamics in 30-minute interval throughout the cotton period were also monitored using CS655 sensors connected to CR-1000 dataloggers from Campbell Scientific. The greenhouse gas chambers and soil moisture sensors will be reinstalled again after cover crop planting in fall 2020 to monitor the emissions and moisture dynamics during the cover cropping period. The measurements will be repeated in the 2021 field season. Soil nutrient dynamics and microbial activity: Soil samples for fertility/plant available nutrients have been taken twice since October 2019. Samples were taken for the 0-15 cm and 15- 38 cm soil depths. Soil samples for controlled incubations were taken once, in March 2020. These samples were taken to assess microbial activity in the top 10 cm profile. Incubations are currently underway to assess general heterotroph activity (as CO₂ respiration), greenhouse gas emission, and carbon, nitrogen, and phosphorus mineralization as a function of the imposed treatments. The results of the analyses from these samples will be correlated against cotton yield and tissue nutrient concentrations (plant uptake). Upcoming operations include the third round of soil fertility sampling and the second round of incubation sampling. Objective 3: Education and outreach Progress: A field demonstration was organized during spring 2020 where nearby farmers interested in transitioning to organic agriculture as well as farmers interested in cover cropping and conservation tillage practices participated. More outreach activities are planned for next year. **PUBLICATIONS (not previously reported):** 2019/09 TO 2020/08 Type: Conference Papers and Presentations Status: Published Year Published: 2020 Citation: Salehin, M.U., Rajan, N., Casey, K., Mowrer, J., Tomlinson, P., Bagavathiannan, M. 2020. Effects of cover crops on greenhouse gas emissions, soil temperature, and soil moisture dynamics in organic cotton. In: Proceedings of the ASA-CSSA-SSSA annual meeting.

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