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HOW DO COVER CROPS CHANGE SOIL HEALTH IN A NO-TILL SYSTEM?

By

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A Thesis Approved on

11/06/2021

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DEDICATION

This thesis is dedicated to my family

Mr. Christopher L. Ross

And children

Aspin, Adin, Astrid, and Ozas

And also my dad

Mr. Stephen J. Tolle

Your support through this process was invaluable

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ABSTRACT

HOW DO COVER CROPS CHANGE SOIL HEALTH IN A NO-TILL SYSTEM?

Aysha K. Tapp Ross
November 9, 2021

The integration of soil health into mainstream agriculture has led to an increase in the use of conservation practices, such as no-till and cover cropping, within the agricultural industry. Nutrient levels and aggregation measurements are currently the most accurate means to measure soil health. However, it has been suggested that bacterial and fungal communities may prove to be a more accurate measure of soil health in the future. In this study soil microbe communities and nutrient levels were compared in rye cover cropped soils to measure for differences between treatments. Effects between the microbial communities and environmental measurements were also measured within those treatments to test for correlations between soil health measures and microbial communities. The plots were put in a biennial corn (*Zea mays L.*) and soybean (*Glycine max*) rotation in 2015, with corn planted in 2020. Cereal rye was examined as the cover crop, compared to bare soil plots, and weedy fallow plots. Available nutrient levels of phosphorus (P), potassium (K), Calcium (Ca), magnesium (Mg), and zinc (Zn) were measured, as well as nitrate levels, ammonium levels, temperature, pH, and volumetric water content (VWC). Bacterial and fungal diversity measures and taxonomic families were compared between treatments. Rye soils were observed to have significantly higher levels of VWC in 2" soil depth while annuals were observed to have significantly higher levels in 6" soil depth. Although there were no significant differences in diversity measures, rye soils had significantly more organisms in the AMF family *Pleosporaceae as well as Helotiales Incertae sedis*, and *Diversisporaceae* fungal families, while bare soil showed an increase in the *Microdochiaceae* family. Further research is discussed to understand possible fungal influence on rye cover cropped soils.

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CHAPTER I
INTRODUCTION

Soil health is an integral part of the sustainable agriculture system (Doran & Zeiss, 2000; Kibblewhite et al., 2008; Tahat et al., 2020). Soil health is a measure of soil stability and is defined by Doran and Zeiss (2000) as “the capacity of a soil to function as a vital living system within ecosystem and land use boundaries to sustain plant and animal production, maintain or enhance water and air quality, and promote plant and animal health”. The United States congress and governmental agencies, such as the National Resources Conservation Services (NRCS), have recently begun focusing on integration of soil health within the agricultural industry through policy (United States Congress, 2021) and utilization (NRCS, n.d.). While there is understanding concerning management practices that increase soil health (Kibblewhite et al., 2008), the mechanisms behind these practices are still largely unknown. While nutrient and aggregation measurements are currently the most accurate means to measure soil health (Wood et al., 2017) it has been suggested that soil microbes, in the form of bacterial and fungal communities, may prove to be a more accurate measure of soil health (Doran & Zeiss, 2000; Tahat et al., 2020). In this study soil microbe communities, nutrient levels, and soil aggregation were compared in rye cover cropped soils to measure for differences between treatments, and to measure effects between the microbial communities and environmental measurements within those treatments to test for correlations between soil health measures and microbial communities.

Background:

Conventional farming relies heavily on the usage of pesticides, herbicides, and synthetic fertilizers (Robertson, 2015). It is best defined by Gold (2007) in a sustainable agriculture report published by the National Agricultural Library and the USDA as a system that shares the characteristics of:

> rapid technological innovation; large capital investments in order to apply production and management technology; large-scale farms; single crops/row crops grown continuously over many seasons; uniform high-yield hybrid crops; extensive use of pesticides, fertilizers, and external energy inputs; high labor efficiency; and dependency on agribusiness. In the case of livestock, most production comes from confined, concentrated systems. (para. 11)

Many of these inputs are responsible for greenhouse gas emissions, terrestrial acidification, and eutrophication of water systems due to nutrient runoff (Poore and Nemecek, 2018). Due to this impact, land practices have become an important aspect of the growing climate change debate.

While farming dynamics are complex, industrialized, or “conventional farming” is largely considered unsustainable (Gold & National Agricultural Library, 2007). The agricultural sector is the fifth largest contributor to the United States greenhouse gas (GHG) emissions, contributing 628.6 MMT CO2 Eq., or 9.6% of the total U.S. GHG emissions in 2019 (EPA, 2021). High input conventional farming contributes substantially to these figures (Arunrat et al., 2021; Sazvar et al., 2018). With global climate temperatures surpassing 1°C above pre-industrial levels in 2017 and continuing to increase (Intergovernmental Panel on Climate Change [IPCC], 2018), the need to decrease GHG emissions is immediate. Low input sustainable agriculture can not only decrease GHG emissions by decreasing fertilizer and diesel fuel use (Lu & Lu, 2017; Shah et al., 2017), but can also be a carbon sink (Stanley et al., 2018)

The need to reduce GHG emissions and nutrient runoff has allowed sustainable agriculture to become an important aspect of the agriculture industry worldwide. In the same sustainability report Gold with the National Agricultural Library (2007) defines sustainable farming in terms of it’s social, economic, and environmental impacts, referring to it as an integrated whole farm system that is capable of maintaining productivity indefinitely. The management techniques utilized in such a system integrate biological cycles and on-farm resources, while minimizing the use of nonrenewable resources (para. 8). Sustainable agriculture provides a means to maintain low-input systems, utilizing integrated management techniques, such as no-till and cover crop management, which work to reduce the damage from conventional farming and improving the health of the soils (Nunes et al., 2018).

The utilization of sustainable agriculture management practices has been suggested as a means to mitigate the negative effects of climate change and increase resilience of our food production systems (Sarkar et al., 2020). Global food systems are not only a major contributor to the growing climate crisis, they are also one of the most vulnerable industries to the projected warming, placing pressures on global food security (Zhao et al., 2017). Projections indicate increases in severe drought conditions and decreases in agricultural yield (Arnell et al., 2019), which would increase food insecurities and disproportionately affect the global poor (IPCC, 2018). Low input agricultural methods may provide essential agricultural adaptation needed to reduce these outcomes, while simultaneously mitigating the agricultural effects on the climate crisis (Sarkar et al., 2020).

Sustainable Agriculture and Policy

There are multiple terms in this discussion of what constitutes sustainable, low input farming, including organic farming, regenerative agriculture, and conservation farming. While each of these methods relies on decreasing the use of synthetic pesticides and herbicides, and increasing natural inputs (USDA, 2011; Schreefel et al., 2020; Hobbs et al., 2008), they each have slightly different meanings. Organic farming is heavily regulated and requires expensive certifications yet provides security to the consumer with the assurance through the Certified Organic badge (USDA, 2011). Regenerative agriculture does not have a clear scientific definition and is not regulated by an institutional entity (Schreefel et al., 2020), making it easy to administer, but difficult to assess. However, within the agricultural and scientific communities it can encompass several management techniques that have been shown to increase soil health, such as no-till, soil cover, and year-round root systems (LaCanne & Lundgren, 2018), to an intensive farming method that integrates livestock and plant diversification that foster soil health and carbon sequestration (Elevitch et al., 2018). Conservation farming, on the other hand, provides a clear policy definition for sustainable farming, one that is regulated and funded by National Resource Conservation Service (NRCS) (Schaible et al, 2015). This allows for a myriad of different techniques that encompass recognized soil health measures of the NRCS without excluding farmers by requiring extensive certifications. It is for this reason that conservation farming will be utilized in discussing sustainable farming further.

Current proposals reflect the need to integrate conservation farming into public policy with bills such as The Growing Climate Solutions Act, which utilizes the United States Department of Agriculture (USDA) in aiding farmers to enter carbon markets (United States Congress, 2021), and increased funding to conservation programs through NRCS (Farm Service Agency, 2021). Kentucky has been working to pass a comprehensive soil health bill (Kentucky General Assembly, 2021) that aims to encourage farmers to move to more climate friendly agricultural methods. Once passed Kentucky will join the few states who have implemented statewide soil health initiatives, such as California (California State Legislature, 2016), Hawaii (Hawaii State Legislature, 2017), Maryland (Maryland State Legislature, 2017), and Oklahoma (Oklahoma State Legislature, 2001).

Through these policies and programs NRCS now recognizes four essential soil health principles that should be implemented by farmers, the same soil health measures defined by regenerative farmers. These principles involve minimizing soil disturbance, implementing soil cover, increasing plant diversity, and continual live roots throughout the year (NRCS, n.d.). Current conservation methods focus on integrating all four soil health measures without requiring excessive management changes for farmers. This is primarily done by fostering the utilization of no-till and cover cropping practices.

Soil Health Practices

Improvements in soil health, by promoting the utilization of healthy soil practices, has been shown to help alleviate the effects of climate change by increasing soil carbon sequestration, and soil's resilience to extreme climate events (Lal, 2016), Soil health practices have also been shown to decrease nutrient runoff and soil erosion, and increase soil biodiversity (Kihara et al., 2020). Healthy soils allow for proper nutrient uptake, water infiltration, and soil microbe communities (LaCanne & Lundgren 2018). Each of these effects helps to alleviate the ramifications of the current agricultural industry.

No till and cover crop management techniques cover all four of the NRCS health soil principles. No-till farming addresses the first principle of minimum soil disturbances with at least 30% of the soil covered in crop residue (Horowitz et al., 2010). Cover cropping addresses the second, third, and fourth principles of healthy soils, that of continual soil cover, increasing plant diversity, and year-round live roots. This method involves planting a crop during periods when cash crops are not on fields, typically over winter. These crops are then terminated prior to planting of the next year’s cash crop (Clark, 2019). This allows continuous planting on fields that would have been bare, added cover of living biomass when growing and residue when terminated, and added plant diversity to crop soils.

While no-till farming has increased to 37% usage in the United States and has become the standard in Kentucky at 68% in 2019 (Zulauf and Brown, 2019), there have been more challenges in implementing cover cropping, with only a 5.7% country wide utilization rate as of 2017 (Wallander et al., 2021). Lower rates of implementation of cover cropping are most likely due to difficulty in navigating management, markets, and weighing the costs of planting and termination (Roesch-McNally et al., 2017). Since these costs of implementing cover cropping deter their implementation, recent studies have focused on soil health benefits, in both conventional and no-till fields, potentially providing benefits that may outweigh costs.

There are multiple options when choosing a cover crop. Cereal rye (*Secale cereale*) is commonly recommended as a cover crop due to its winter hardiness (SARE, 2007) which allows it to be planted later in the season than other cover crops. Rye, at a seeding rate of 2 bushels per acre, also provides weed suppression by reducing weed biomass over other cover crops, such as hairy vetch (Zotarelli et al., 2009). Currently the amount of weed suppression is not adequate to eliminate the need for herbicides (Malik et al., 2008). However, higher seeding rates of up to 6 bushels per acre may provide adequate weed suppression (Sustainable Agriculture Research and Education [SARE], 2007). Rye also uptakes excess nitrogen, preventing leaching during winter months (Kaspar et al., 2007). While cereal rye may cause a decrease in corn yield, which could be a result of allelopathic effects of rye, changes in moisture, or pathogens (Patel et al., 2019), it is an optimum cover prior to a soybean rotation, especially since soybeans are a nitrogen fixing legumes. Rye also fosters arbuscular mycorrhizal fungi (AMF) communities (Gollner et al., 2011) which help regulate nutrient cycling and plant productivity (Mendes et al., 2015).

The soil health benefits of conservation agriculture involve increased carbon sequestration through the use of no-till (Bai et al., 2019) and cover crop utilization (McDaniel et al., 2014; Poeplau and Don, 2015). These methods also increase soil microbial biomass (di Rauso Simeone et al., 2020), including AMF (Thapa et al., 2021), and bacterial communities (Navarro-Noya et al., 2012). In recent years studies have shown that increased soil carbon in these conservation methods may be due in part to the increase in AMF (Gottshall et al., 2017), and bacteria (Mau et al., 2014). However, fungi use carbon more efficiently and form more biomass per unit of carbon than bacteria (Gougoulias et al., 2014), which may mean that fostering healthy fungal communities may be more sustainable.

Increased fungal biomass, or measure of the mass of the soil fungal microbes present, in agricultural soils has also been shown to increase nitrogen retention (de Vries et al., 2011). Fungal and bacterial community functioning is essential to proper nitrogen uptake (Emmett et al. 2020; Mensah et al. 2015). Nitrogen, in the form of organic molecules, is broken down by bacterial and fungal decomposers by means of ammonification into ammonium (NH_4^+). Atmospheric nitrogen (N_2) is also converted into NH_4^+ by means of nitrogen-fixing bacteria, such as Rhizobia found in the nodules of specific perennial legume roots. Nitrifying bacteria then convert ammonium into nitrites (NO_2^-), and then nitrates (NO_3^-) through nitrification, when it can then be absorbed by flora (Stein & Klotz, 2016). Through this process the fungal and bacterial communities play a crucial role in nitrogen uptake, especially considering nitrogen is often a limiting factor in plant growth (Vitousek et al., 2002).

Increased microbe biomass levels are beneficial to agricultural systems; however, an equally important microbial measure is soil biodiversity, or the measure of variability in soil microbes. Soil biodiversity has also been shown to improve plant diversity, decomposition, nutrient retention, and nutrient cycling (Wagg et al., 2014). Soil microbial diversity has been shown to increase plant productivity (Raynaud et al., 2021; Reynolds et al., 2003), and the decreasing of soil microbial diversity increases the risk of plant diseases (Schnitzer et al., 2011). Increasing soil microbial biodiversity is also important for soil functioning, and agricultural conservation methods can reinforce soil ecosystem services, such as carbon and nutrient cycling and pest control (Thiele-Bruhn et al., 2012). This is done in part by stimulating organic phosphorus cycling (Hallama et al., 2021), regulating nutrient cycling and plant pathogen defense (Wetzel et al., 2014), and playing a role in plant uptake of P, Fe, Zn, Mn, and Cd (Hontoria et al., 2019), making soil microbial diversity as important an indicator of soil health as biomass. It is apparent that microbial diversity in agricultural soils is paramount in ensuring proper soil health, which promotes carbon sequestration, increased nutrient retention, and increased plant health.

Studies into whether conservation practices in agriculture increase soil microbial diversity have increased since the need to sequester carbon emissions and decrease nutrient runoff has become of vital importance. These diversity readings may end up being as important a soil health measure as aggregate, nutrient, and infiltration measures currently utilized in agriculture. With fungi and bacteria working in a mutualism with agricultural crops it is important to focus on conservation methods which increase these biodiversity levels. With this knowledge we can influence policy to encourage the utilization of these practices to help transform the agricultural sector into a more sustainable system.

Soil Health Study

While there has been evidence that conservation farming methods of no-till and cover cropping increases fungal (Chen et al., 2013) and bacterial (Leite et al., 2021; Tyler, 2019) biomass, there is contradicting evidence that these methods also increase fungal and bacterial biodiversity. In this study soil fungal and bacterial diversity were measured, along with their relationship to soil nutrient levels, bulk density, VWC, and temperature measures in a long-term no-till study with differing cover crop treatments in Central Kentucky. Cereal rye was examined as the cover crop, compared to bare soil plots, sprayed with herbicides in the fall to prevent weed growth, and weedy fallow plots, which were not sprayed with herbicide to promote growth of annual winter weeds. Available nutrient levels of phosphorus (P), potassium (K), Calcium (Ca), magnesium (Mg), and zinc (Zn) were measured, as well as nitrate, ammonium levels.

Hypothesis 1:

> Soils with a cereal rye cover crop would promote higher fungal and bacterial diversity.

Hypothesis 2:

> Rye cover crop plots would have lower bulk density and higher nutrient availability relative to bare soil and a cover of weedy annuals.

Hypothesis 3:

> Rye plots would have an effect on microbial composition. In particular, fungal and bacterial families would show differentiation between rye and cover crop plots.

CHAPTER II
METHODS

Long term field experiment

The experimental field was part of a long-term cover crop study located at a field site at the University of Kentucky Spindletop Research Farm located in Lexington, Kentucky. Lexington is located in the Bluegrass region and yields yearly average highs of 18.3°C, lows of 7.78°C (U.S. Climate Data, 2021), and has an annual precipitation of 49.84” (National Weather Service, 2021). Three treatments were measured, with four replicates per treatment, for a total of twelve total plots. Plot sizes were 30’ x 80’ and were arranged into four neighboring fields, each field containing one of each treatment randomly assigned to one of four plots within the field (Figure 1).

The plots were put in a biennial corn (*Zea mays L.*) and soybean (*Glycine max*) rotation in 2015, with the most recent harvesting of corn on October 23, 2020. Prior to the planting of corn. All treatments were under no-till management. Annual and rye plots were sprayed with Dicamba herbicide each year post cover crop termination, to prevent growth of residual weeds, prior to corn or soy seeding. The three treatments were bare soil, weedy fallow, and cereal rye. The bare soil treatment was sprayed with a Dicamba herbicide in the fall of each year to prevent weeds growing over the winter period, with spraying occurring on November 5, 2020. The weedy fallow (annual) plots were not sprayed with herbicide prior to winter, which allows for the growth of winter annual weeds. Cereal rye was utilized as the overwintering cover crop. Prior to corn planting the rye plots were cover cropped in a crop mixture of rye and hairy vetch (*Vicia villosa*). A cover crop of rye was planted prior to planting of soybeans. Cereal rye was planted on October 27, 2020 at a seeding rate of 100 lbs. acre^{-1}. Soil samples were collected on April 7, 2021.

Soil sampling

Samples of each of the twelve plots were taken by transecting each plot vertically and horizontally through the middle of each plot. Each soil sample was collected with a .39” wide by 2.36” deep soil probe at ten feet intervals along the length and width for a total of 9 samples and homogenized in a gallon freezer bag (Figure 2). Soil was collected from each homogenized sample into a sterile 15mL centrifuge tube and placed on dry ice. The homogenized samples were sent to Omega Bioservices (Norcross, GA) for sequencing. High throughput sequencing (IIlumina MiSeq) was used to analyze bacterial and fungal community composition utilizing 16s rRNA and ITS1 gene reamplification. Bulk density was sampled using a 4.25” golf hole corer to a depth of 4”, dried at 105℃, and weighed.

Soil moisture, nutrient data, and pH levels from 2020 were obtained, as well as nitrogen levels from 2019 and 2020 (Haramoto, personal communication, July 9, 2021). Soil inorganic nitrogen was extracted through .35oz of soil in .85 fl oz of 1M KCl. Nitrate and ammonium levels were recorded at ppm. Each measurement was obtained for both the spring and fall of 2019 and 2020. P, K, Ca, Mg, and Zn, and pH were obtained by standard soil test run by regulatory services at the University of Kentucky.

Soil-water pH was used for pH level as soil-water pH is more closely related to optimum plant growth (Cooperative Extension Service, 2020). Soil temperature and volumetric water content were recorded via soil moisture sensors placed at 2” and 6” depths. The sensors took readings every hour and were continually logged. The study utilized daily averages from the month prior to sampling, starting on 3/1/2021 and ending 4/7/2021.

Photos were taken of each plot on the same day the soil samples were processed (Figure 3). Plant cover and species representation were not taken the day of soil collection. They were analyzed via the pictures at a later date.

Statistical analysis

Data were analyzed using RStudio (version 1.4.1717) running R version 4.1.1. The number of organisms in individual fungal and bacterial families were analyzed when organisms within families were ≥500 in any of the twelve plots. The organism number in each family was then measured against treatment types. All fungal family data that were analyzed were log transformed in order to fit a normal distribution, except *Diversisporaceae* which utilized the square root transformation. General linear model ANOVA tests were run using lmerTest package with number of organisms in a family as a numerical response variable, also with plot replicates as a random effect to account for interractions across replicated plots. When main effects were significant, post-hoc pairwise comparison tests using Tukey's HSD were performed.

Nutrient content for P, K, Ca, Mg, Zn, ammonium, and nitrate were compared against treatment type via general linear model tests, with concentration levels as a numerical response variable, also with plot replicates as a random effect to account for interactions across replicated plots. Ammonium and nitrate were also compared against year and season as categorical variables. Bulk density and soil water pH was also compared in the same manner with either the bulk density, or the soil water pH, as the numerical variable.

Volumetric Water Content and temperature readings were analyzed using the overall means of the daily means per plot. They were compared by treatment type via a linear mixed model with either moisture or temperature reading as a numerical response variable, a categorical variable of treatment, and a categorical variable of depth, with plot replicates as a random effect to account for interactions across replicated plots. When main effects were significant, post-hoc simple two-way interaction ANOVA tests were performed with a Tukey's HSD.

Diversity measures were compared over fungal and bacterial data. Fungal and bacterial evenness, richness, Shannon Diversity, and total organisms measured were analyzed. Shannon diversity was calculated by Omega Bioservices using the formula:

$$H' = -\sum((p_i) * \ln(p_i))$$

Where p_i= number of individuals of species/total number of samples. Evenness, the measure of how uniform each number of organisms are for each species in a community, was calculated using the formula:

$$E=H'/H'max$$

$$H'max = \ln(N)$$

Where N is the richness, or total number of species. The data was analyzed using general linear models with diversity or organism measures as a numerical variable against treatment, with plot replicates as a random effect to account for correlated values across replicated plots.

Nutrient data, bulk density, pH, VWC, and temperature were analyzed using a principal components ordination analysis (PCA) in R with the vegan package (Oksanen et al., 2019). Bacterial and fungal family community structure was also examined using a non-metric multidimensional scaling (NMDS) ordination analysis to observe dissimilarities in treatments through distance.

CHAPTER III
RESULTS

Effects on nutrient content, moisture levels, and bulk density

There were no significant differences between treatments in the nutrients tested, P, K, Ca, Mg, and Zn, or bulk density readings (model effect of treatment: Table 1, Figure 4). Soil water pH was log transformed to fit a normal distribution; no significant difference was observed. Log transformations were performed on nitrate data to fit normality. Significant differences in nitrates were recorded between seasons, with nitrates measuring higher in the fall ($p=0.0004$,), and seasons between years, with the fall of 2019 measuring higher than spring of 2019 ($p=5.09e-06$) and both spring and fall of 2020 ($p = 6.88e-07$). Statistically significant differences were not found between treatments (model effect of treatment: Table 2, Figure 5). Outliers in ammonium data were included when running linear models, but not in figures. An exponential transformation was performed on ammonium data to fit a normal distribution. Ammonium did not show statistically significant differences between treatment ($p=0.166$), year ($p=0.337$), or season ($p=0.158$).

In plotting the treatment means of the daily VWC means there seems to be a trend for the VWC treatments (Figure 6). In testing for effects, VWC data was square transformed (x^2) to fit a normal distribution and was run through a linear model. Table 3 shows interactions from the linear mixed model analysis of variance indicating a statistical difference for VWC between treatments (p= 3.57e-16), between depths (2.2e-16), and between treatment and depth (2.2e-16). Depths were isolated and ad hoc Tukey's HSD testing was performed on each depth. It was determined that the significant difference in 2" depths between treatment was between both rye and bare soils (p=.0001), and rye and annuals (p=.000001), indicating that the VWC for cereal rye was higher than for the bare soil and annual plots. Significant differences in 6" depths were between annuals and bare soil (p= <2e-16) and annuals and rye (p= <2.2e-16), indicating that VWC for annual plots was higher than in bare soil or cereal rye plots.

The temperature readings did not show statistically significant differences over treatments and depths (model effect of treatment: Table 4, Figure 8). Although depth differences between 6" and 2" had a p value of 0.057.

Ordination plots demonstrated that the standard deviation ellipses for treatments were overlapping, and thus were not demonstrating a difference in environmental factors throughout the treatments (Figure 9).

Effects on fungal and bacterial communities

Shannon Diversity, evenness, richness, and number of individuals were analyzed for fungal and bacterial readings using data obtained from Omega Bioservices. Means and standard deviation were assessed (Table 5). In analyzing for differences in diversity measures, there were no significant differences (Table 6, Figure 10)

The number individuals within the fungal families *Pleosporaceae*, *Helotiales Incertae sedis*, and *Diversisporaceae* were affected by treatment, and post hoc testing indicated there were statistically significant differences in cereal rye than the other treatments (Table 7, Figure 11). Bare soil displayed a statistically significant difference in number of organisms in the fungal family *Microdochiaceae* (Table 7, Figure 12, p=.003). There were no significant differences in bacterial families between treatments.

Non-metric multidimensional scaling (NMDS) plots, utilizing the standard deviation of the point scores for each treatment as ellipses, indicated that bacterial communities differed between cereal rye and annual plots, and bare soil and annual plots, but not cereal rye and bare soil (Figure 13), while there was no difference between fungal community composition and treatments (Figure 14)

Plant Composition and Growth

Annuals were also present in both the annual plots and the cereal rye plots. Purple dead nettle (*Lamium purpureum)* and the common violet (*Viola sororia)* (Figure 15) was the most abundant in annual plots 102 and 201, and in the cereal rye plots 104, 204, and 304, while the perennial dandelions (*Taraxacum officinale*) were present in plots 301 and 404.

CHAPTER IV
DISCUSSION

The abundance of AMF taxa *Diversisporaceae* indicate an association between cereal rye and their fungal communities, which is typically found in organic soils, managed under a moldboard plough tillage, with no pesticides or herbicides (Banerjee et al., 2019). Even though rye is a known AMF host, it is still promising to see an increase in beneficial AMF communities in the rye soils, especially during vegetative growth early in the season when AMF abundance is shown to be higher than later in the season (Welsh et al., 2010). Previous studies have also shown that increasing AMF abundance in agricultural soils can negatively affect the growth of some weed species (Veiga et al., 2011), and some weeds negatively affect the presence of AMF fungal communities (Wortman et al., 2013), however since weed samples were not collected it is unknown whether these weeds occurred in the plots.

AMF communities have been shown to support plants in nutrient acquisition (Debeljak et al., 2018), contribute to soil aggregate formation (Leifheit et al., 2014) and protect their host plants from environmental stresses (Bunn et al., 2009; Chen et al., 2013). The utilization of rye as a cover crop has even been shown to promote mycorrhizal fungus colonization of the subsequent crops (Kabir and Koide, 2002). Therefore, utilization of rye as a cover crop has the potential to increase root functioning in the cash crops following rye implementation, which could increase yield.

The presence of the family *Pleosporaceae* however, is troubling. *Pleosporaceae* are bitunicate ascomycetes that are known pathogens, including species that cause corn leaf blight (*Cochliobolus heterostrophus*) and disease in cereals (*Bipolaris maydis*, *Setosphaeria turcica,* and *Cochliobolus miyabeanus*) (Kodsueb et al., 2017). However, none of these specific species were present in any of the field plots. While the species within the *Pleosporaceae* family are considered to be pathogens, some do so by acting as parasitic endophytes, which can be used for weed control (Kia et al., 2016), meaning that they could be acting as a weed suppression instead of possibly damaging crops. *Helotiales Incertae sedis* are species of the order *Helotiales* that have not yet been placed in a family, and they are difficult to classify (Johnston et al., 2019). Further examination is needed to identify the possible effects of both families within rye soils.

Microdochiaceae are endophytes thought to be important members of fungal communities in harsh, nutrient-limited environments (Knapp et al., 2019). Their presence in bare soils indicates a possible mutualistic benefit for the crops grown in these environments. The abundance of this family in bare soils over annual or rye soils may provide insight into soil functioning of conventional farming methods.

The significant difference in nitrates between the spring and fall of 2019 was most likely due to an increase in nitrate levels in the fall of 2019 following a soybean crop rotation. Soybean is a legume, which are nitrogen fixers, and their presence increases available nitrogen within soils (Zahran, 1984).

The Shannon Diversity (H') readings for fungal and bacterial communities were similar to those in other studies. The fungal H' measures for rye (H' =3.79) and bare soil (H'=3.61) were higher than Schmidt et al. (2018) in comparing cover cropped (H'=2.55) to no cover (H'=2.76) plots. They were also higher than Hontoria et al. (2018) in their experimental no till plots with Barley (H'=1.92) as a cover crop, and bare soil (H'=1.72). Sommerman (2018) investigated tillage effects on fungal diversity and showed higher results of H'=3.9 in no tilled soils. The cover crop studies also did not show significant differences in H' for cover cropped soils.

Bacterial H' was lower for rye (H'=4.42) and bare soil (H'=4.45) than in other cover crop studies. In no-till soils bacterial H' was 6.1 (DeGrune et al., 2016). Buckwheat cover crops in tilled soils displayed H' = 9.58 directly before termination of the cover crop, while in no cover plots H' = 9.37 (Wang et al., 2020). The differences in H' could be due to the management of the soils prior to the experiment, Wang et al. utilized organic soils, however there is little evidence that organic systems increase bacterial diversity (Armalytė et al., 2019; Palma-Cano et al., 2021).

Even though soil microbe diversity was not significantly different between treatments there are several reasons why this might have occurred. Other studies have shown the implementation of no-till practices influence soil microbial communities (Dong et al., 2017; Sharma-Poudyal et al., 2017) even over cover crop utilization (Tyler, 2020). This indicates that cover crops are beneficial on tilled soils, but perhaps not as much on no-till soils.

Timing of soil samples has been shown to influence diversity and community measures. Nevins et al. (2018) demonstrated that variation in microbe communities decreases and becomes more specialized starting 21 days after cover crop termination, with the presence of cover crop residue. Testing the soils again after cover crop termination may have demonstrated different microbe community variation and diversity results.

Evidence suggests that covering soils in plant residue increases soil microbe diversity (Bending et al., 2002, Govaerts et al., 2007), it is also hypothesized that continuous covering of soils with crop residues, such as crimping rye prior to planting, is even more beneficial (Romdhane et al., 2019). Even though cover crop residues remained on the treatment soils, both the cereal rye and annual plots have large sections of plots where cover did not grow. It is possible the data did not show a significant difference because the biomass of the living plant cover was too low to make a difference in soil microbe levels.

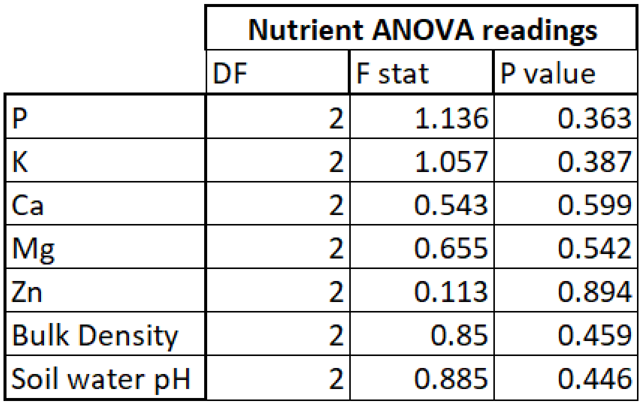
Cover crop management may also play a factor in soil microbe diversity. Few studies have investigated the differences in how cover crops are managed; whether the cover crop is sprayed, removed, or crimped before planting of the successional crop, and which cover crops are used. It is possible that soil coverage after cover crop termination matters just as much as the utilization of cover crops. Cover crop residue was left on the fields prior to termination via herbicide throughout the study. However, while maintaining cover crop residue preserves fungal and bacterial diversity (Navarro-Noya, 2012) the use of herbicides does not (Vukicevich et al., 2016), potentially causing microbe communities to remain similar to bare soil plots, despite the addition of beneficial cover crop residue. Finally, it has been indicated that there are species-specific cover crops effects on microbial communities that influence soil biological activities (Finney et al., 2017) indicating that the utilization of non-rye cover crops, or a mixed cover crop, should demonstrate alternative community differentiation.

While cereal rye cover crops are a weed suppressant, they are not sufficient in eliminating the need for herbicides. Thus, rye cover crop management in Kentucky relies on herbicide even with the weed suppression it provides (Sherman et al., 2020). It is recommended that rye be seeded at 1-2 bushels acre^{-1} in late fall and terminated by tillage or herbicides in early spring, at eight to twelve inches in height (Rector et al., 2009). However, Sustainable Agriculture Research and Education (SARE) suggests seeding up to 6 bushels acre^{-1} for weed management with the use of a roller crimper for termination (2007), indicating a potential for weed suppression without the utilization of herbicides, although more testing is needed in this area.

While many of the statistical analyses did not show significant differences between treatments this may have been due to a type II statistical error. Type II errors occur when a null hypothesis, that there is no significant difference between treatments, fails to be rejected because the statistical tests do not hold enough power. Only having four data points per treatment does not give much power to a statistical test. In order to counteract this in the future there either needs to be more replicates, or more data taken over longer periods of time.

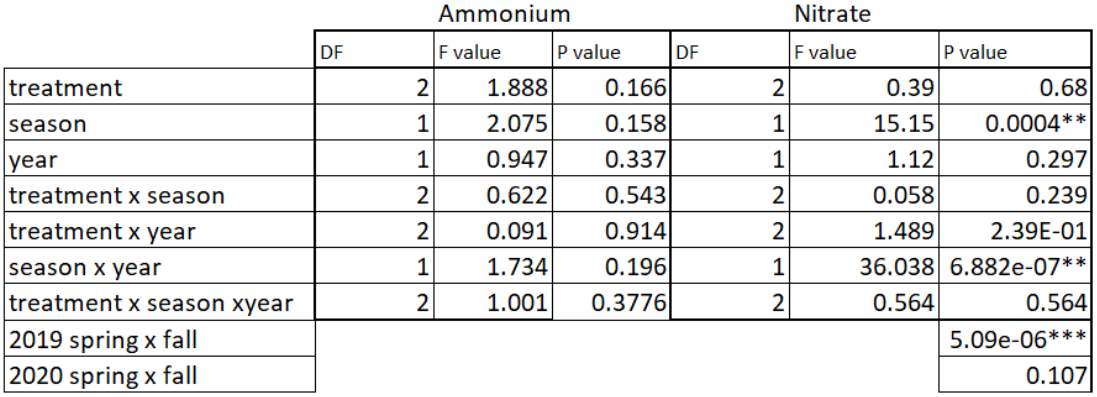
While there is conflicting evidence of the effects of cover crops on fungal and bacterial communities in no-tilled soils, the presence of increased AMF communities in cereal rye soils could indicate increased soil health. Further soil testing on the plots 21 days or more post cover crop termination with residue presence would be needed to assess further microbe specialization within the soils, preferably with herbicide use as a variable. Testing should also occur over multiple seasons to assess for effects of rye biomass if decreased biomass did have an effect this year. While it is understood that cover crop utilization is beneficial in tilled soils, the benefits on no-tilled soils still needs further study. However, the potential increase in AMF communities in cover cropped soils is promising and could potentially aid in the increase of soil aggregation, nutrient uptake, and decrease in environmental stresses in agricultural soils.

It is possible that by exploring microbial communities within agricultural soils we can develop and promote a better understanding of proper conservation agriculture methods that promote soil health. By understanding the mechanisms behind nutrient uptake, water infiltration, and most importantly carbon sequestration within agricultural soils we can help steer the direction of conservation programs that will inevitably be influencing agricultural carbon markets and other climate related policy programs. With this influence we could provide a more concrete method to ease climate change pressures on the agricultural and food industries in subsequent years.



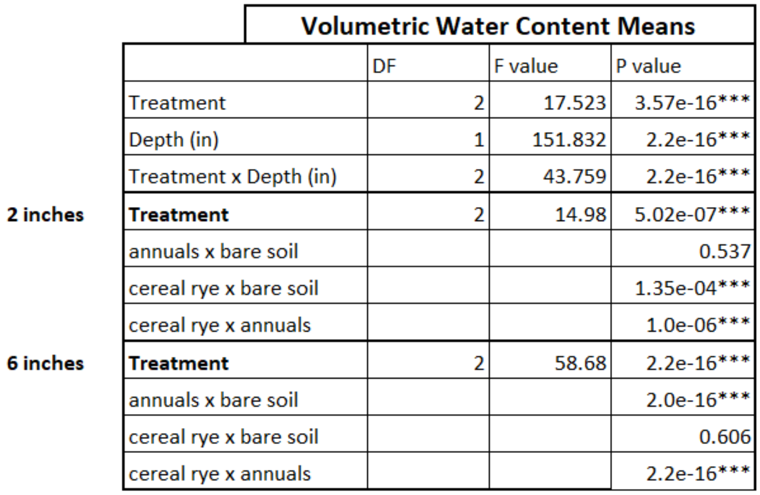
	Nutrient ANOVA readings		
	DF	F stat	P value
P	2	1.136	0.363
K	2	1.057	0.387
Ca	2	0.543	0.599
Mg	2	0.655	0.542
Zn	2	0.113	0.894
Bulk Density	2	0.85	0.459
Soil water pH	2	0.885	0.446

Table 1: General linear model results for nutrients, bulk density, and soil water pH. No significant differences were found.



	Ammonium			Nitrate		
	DF	F value	P value	DF	F value	P value
treatment	2	1.888	0.166	2	0.39	0.68
season	1	2.075	0.158	1	15.15	0.0004**
year	1	0.947	0.337	1	1.12	0.297
treatment x season	2	0.622	0.543	2	0.058	0.239
treatment x year	2	0.091	0.914	2	1.489	2.39E-01
season x year	1	1.734	0.196	1	36.038	6.882e-07**
treatment x season xyear	2	1.001	0.3776	2	0.564	0.564
2019 spring x fall						5.09e-06***
2020 spring x fall						0.107

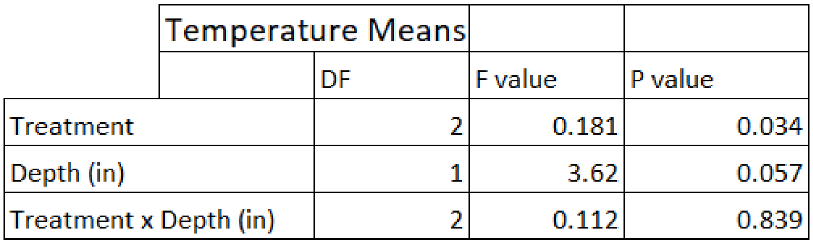
Table 2: General linear model table for ammonium and nitrate values. Significant differences were found between seasons and between seasons and year. Ad hoc testing determined the significance was with the fall of 2019.



Volumetric Water Content Means

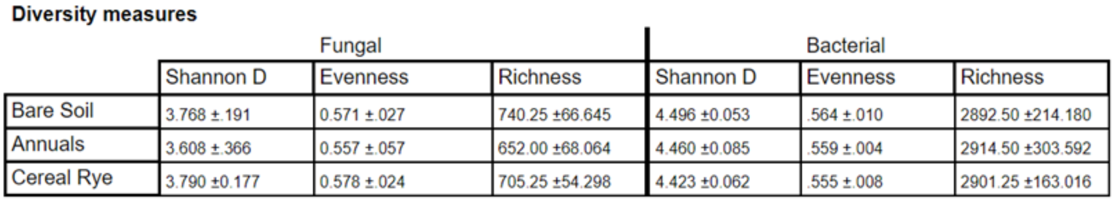
		DF	F value	P value
	Treatment	2	17.523	3.57e-16***
	Depth (in)	1	151.832	2.2e-16***
	Treatment x Depth (in)	2	43.759	2.2e-16***
2 inches	**Treatment**	2	14.98	5.02e-07***
	annuals x bare soil			0.537
	cereal rye x bare soil			1.35e-04***
	cereal rye x annuals			1.0e-06***
6 inches	**Treatment**	2	58.68	2.2e-16***
	annuals x bare soil			2.0e-16***
	cereal rye x bare soil			0.606
	cereal rye x annuals			2.2e-16***

Table 3: General linear model values for volumetric water content. Significant differences were found between treatments and between depth. Depths were isolated and ad hoc Tukey’s HSD tests were run on 2 inches and 6 inches. In 2-inch levels cereal rye showed significant differences from bare soil and annual plots. In 6-inch levels annuals showed significant differences from bare soils and rye



Temperature Means			
	DF	F value	P value
Treatment	2	0.181	0.034
Depth (in)	1	3.62	0.057
Treatment x Depth (in)	2	0.112	0.839

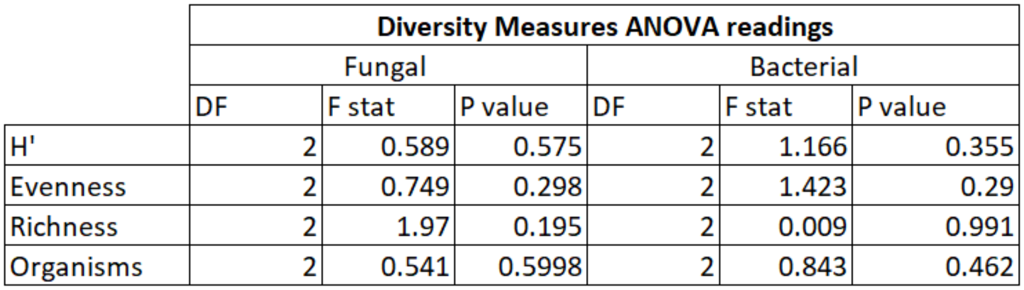
Table 4: Temperature general linear model results. No significant differences were recorded between treatment or depth.



Diversity measures

	Fungal			Bacterial		
	Shannon D	Evenness	Richness	Shannon D	Evenness	Richness
Bare Soil	3.768 ±.191	0.571 ±.027	740.25 ±66.645	4.496 ±0.053	.564 ±.010	2892.50 ±214.180
Annuals	3.608 ±.366	0.557 ±.057	652.00 ±68.064	4.460 ±0.085	.559 ±.004	2914.50 ±303.592
Cereal Rye	3.790 ±0.177	0.578 ±.024	705.25 ±54.298	4.423 ±0.062	.555 ±.008	2901.25 ±163.016

Table 5: Mean and standard deviations for diversity measures in bacterial and fungal communities



	Diversity Measures ANOVA readings					
	Fungal			Bacterial		
	DF	F stat	P value	DF	F stat	P value
H'	2	0.589	0.575	2	1.166	0.355
Evenness	2	0.749	0.298	2	1.423	0.29
Richness	2	1.97	0.195	2	0.009	0.991
Organisms	2	0.541	0.5998	2	0.843	0.462

Table 6: General linear model results for diversity measures for fungal and bacterial data. No significant differences were recorded.

Fungal Families

	Bare x Annuals	Bare x Rye	Rye x Annuals	DF	F value	P value
Pleosporaceae	0.48	0.007**	0.001**	2	15.583	0.001**
Helotiales Incertae sedis	0.486	0.002**	0.011**	2	13.56	0.002**
Diversisporaceae	0.36	0.000006**	0.00002**	2	64.697	4.561e-06**
Microdochiaceae	0.007**	0.085	0.312	2	8.152	0.0095**

Table 7: General linear model results for fungal family data. Significant differences were found for *Pleosporacae, Heliotiales Incertae sedis,* and *Diversisporaceae* between bare soil and cereal rye, and cereal rye and annual plots. Also significant differences were found for *Microdochiaceae* between bare soil and annuals.

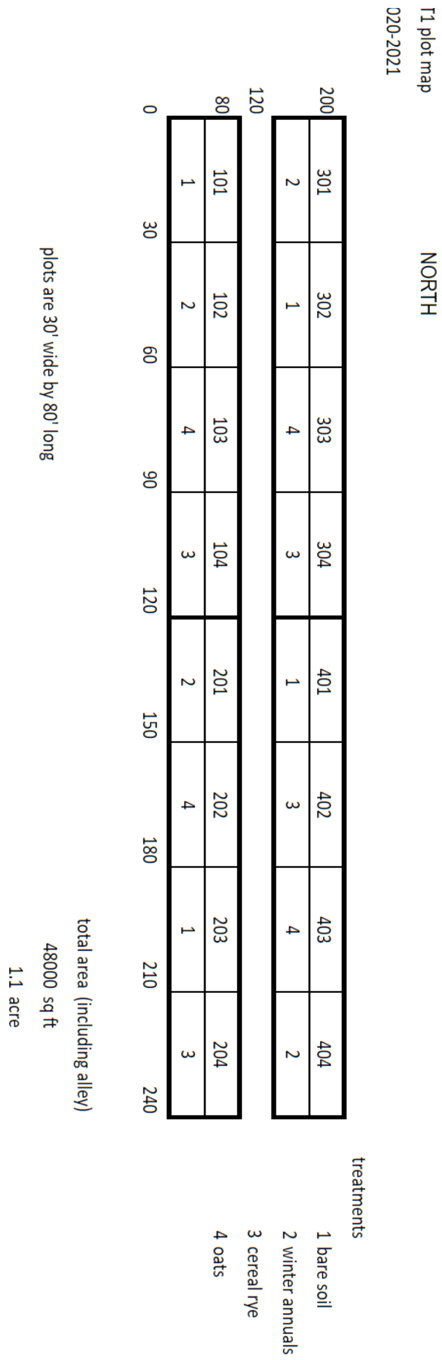


Figure 1. Map of Spindletop Farm fields with treatment plots. Created by Dr. Erin Haramoto. We did not sample treatment 4, oats.

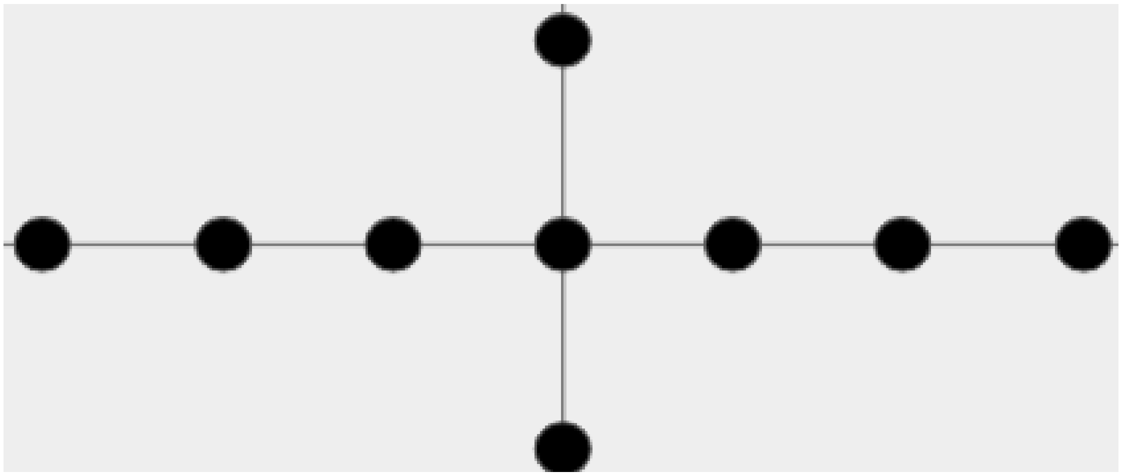


Figure 2. Transects of each 30'x 80' plots. Samples along the 30' length were taken every 10 feet. Samples along the 80' width were taken every 10 feet.

Treatment 1: Bare Soil

Treatment 2: Annuals

Treatment 3: Cereal Rye

Figure 3. Pictures of the individual plots

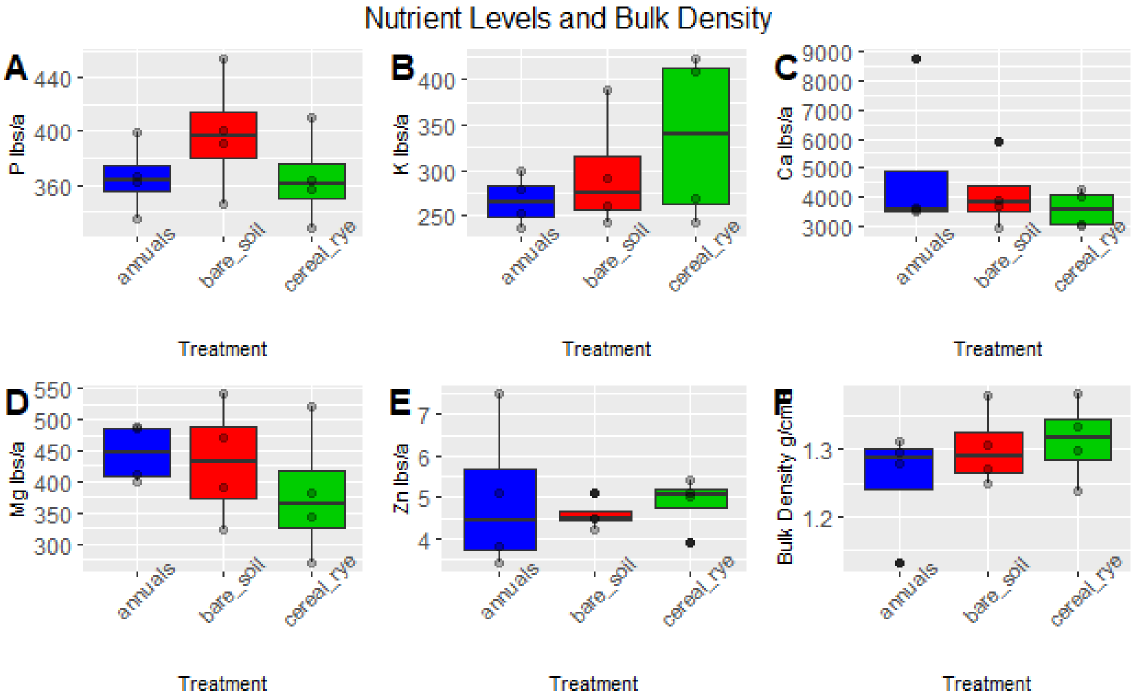


Figure 4. Nutrient levels and bulk density readings between treatments.

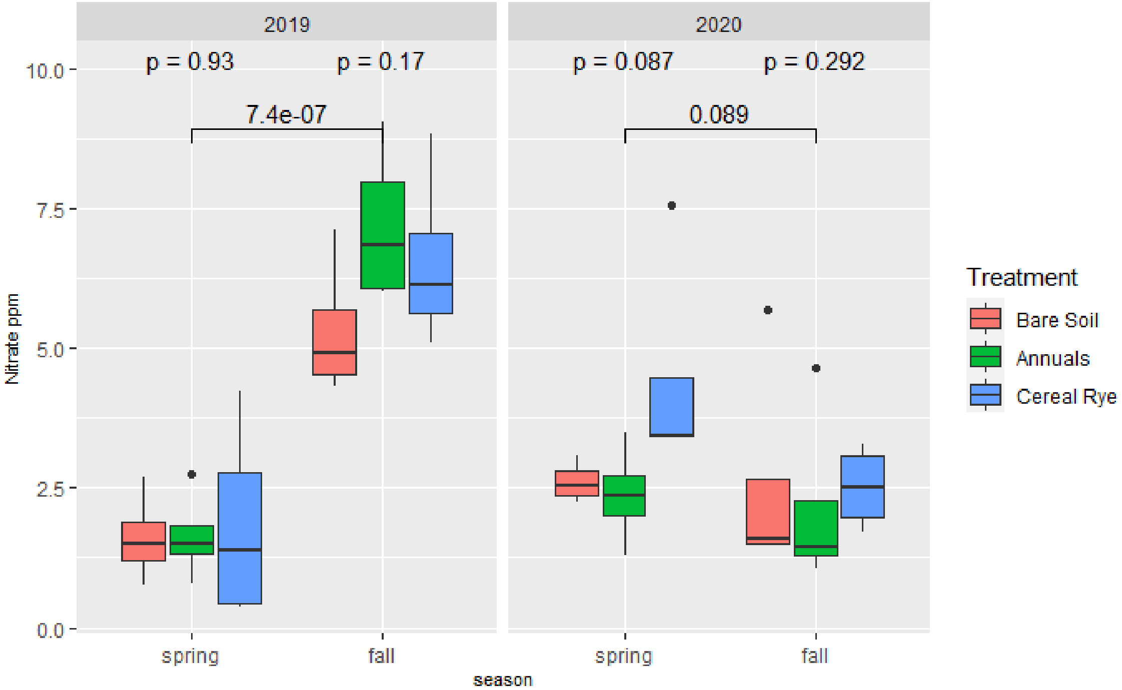


Figure 5. Nitrogen levels between spring and fall of 2019 and 2020 with respect to treatment, with p values for treatment per season and between seasons in each year.

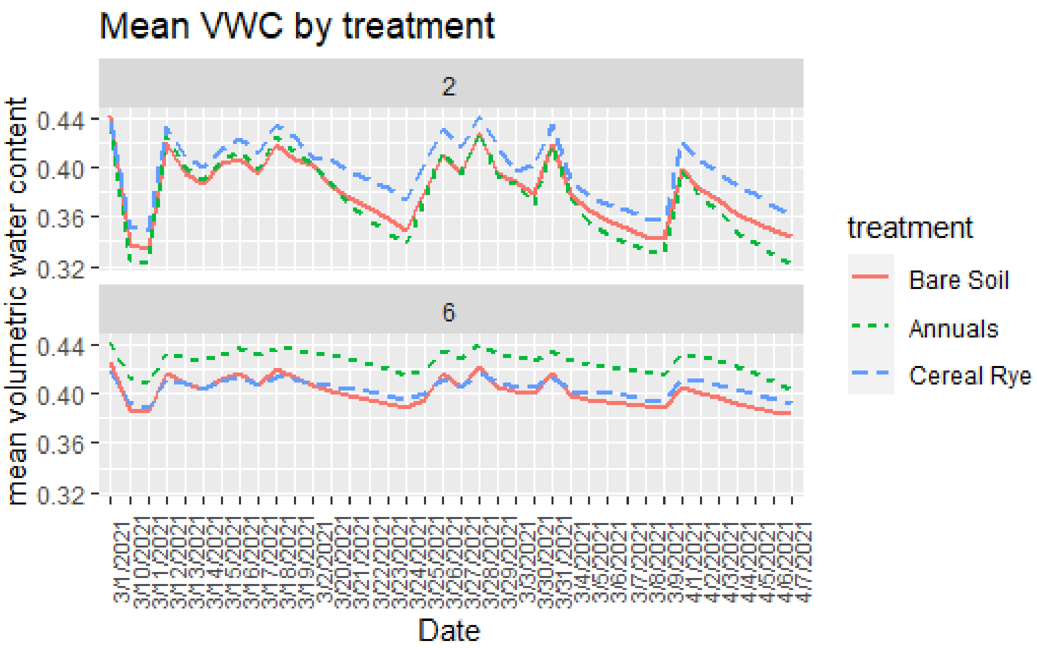


Figure 6. Mean values for the mean VWC per treatment per day in 2in and 6in depths.

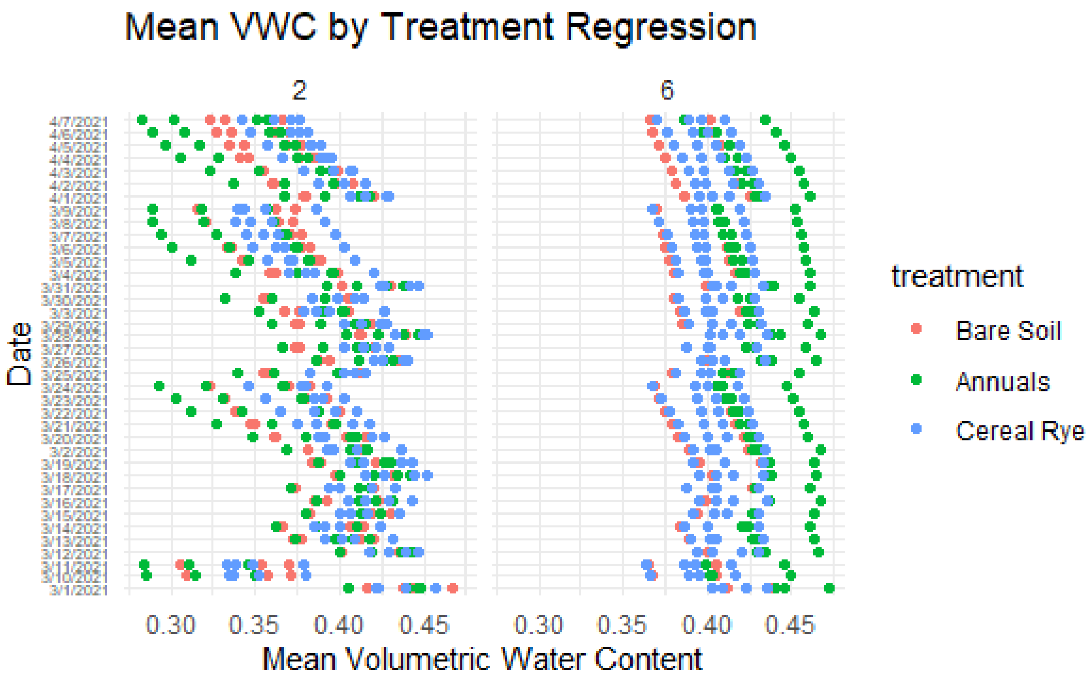


Figure 7. Mean volumetric water content per plot per day over 2in and 6in depths

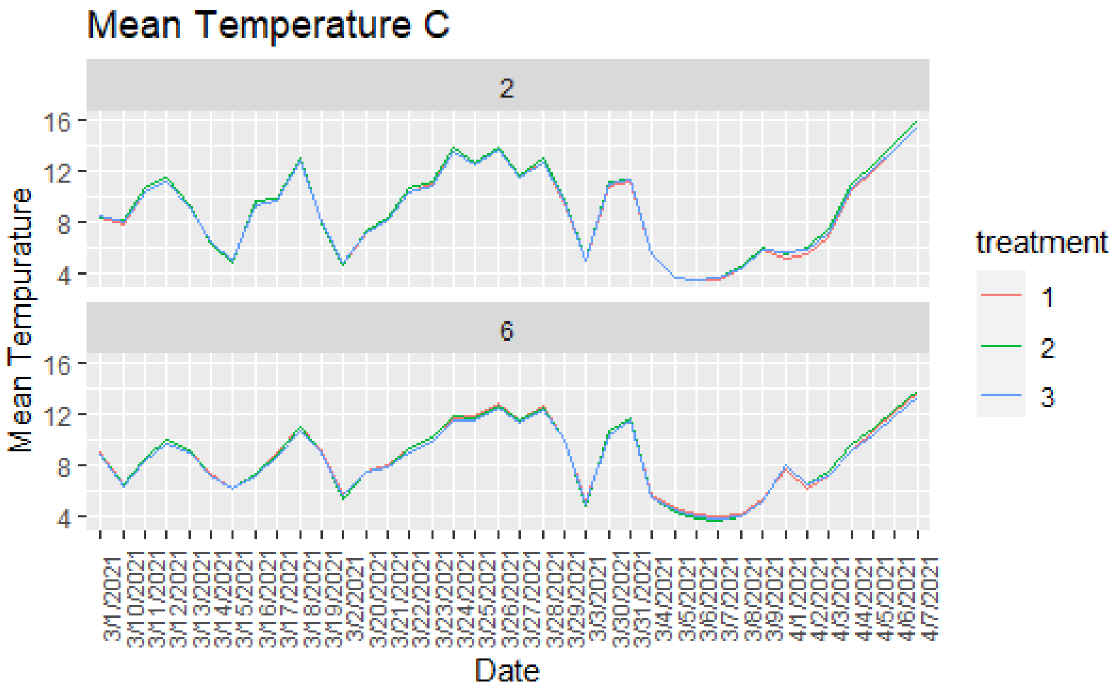
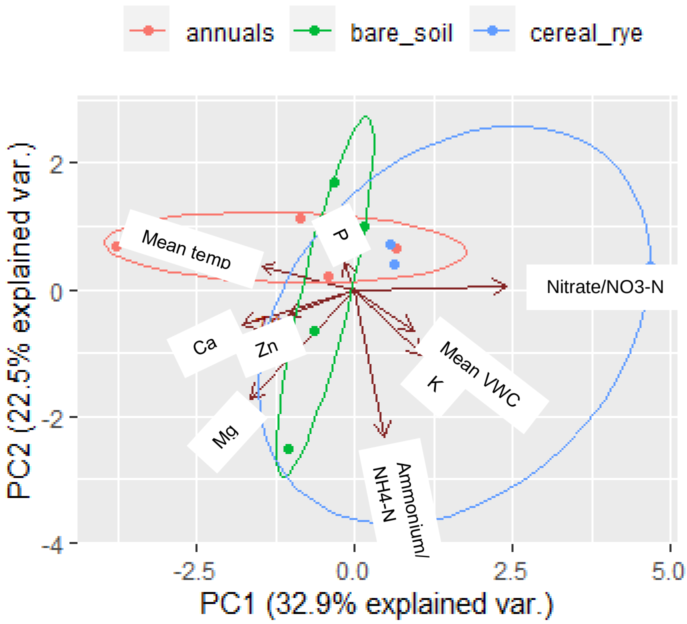


Figure 8. Mean values for mean temperatures per day per depth in relation to the three treatments.



Mean

temp

Figure 9. Ordination plots from the redundancy analysis (RDA) results exploring the relationships between environmental factors (nutrients, temp, VWC, and nitrogen) and treatment plots±.

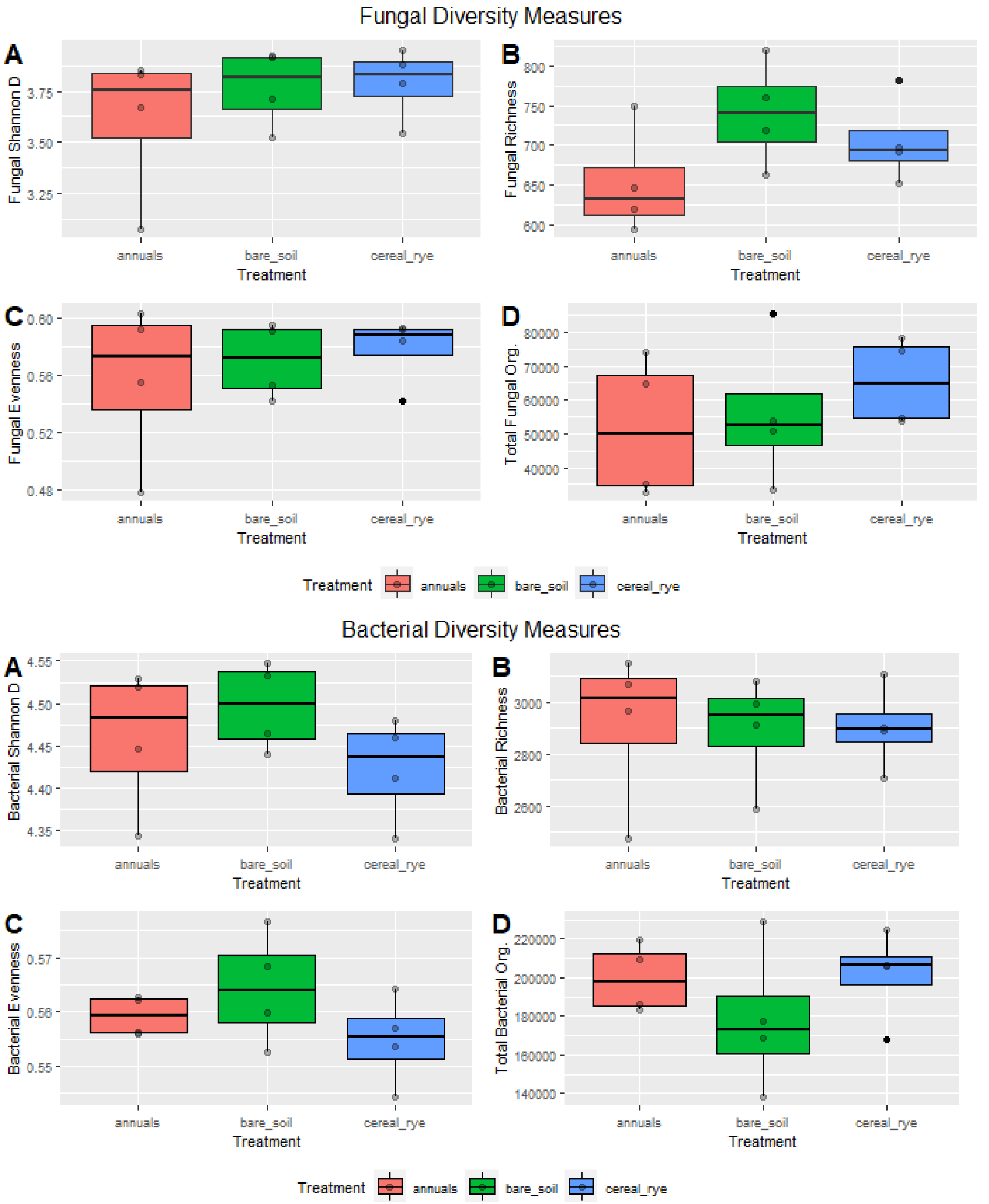


Figure 10. Fungal and Bacterial diversity measures: Shannon D (A), Richness (B), Evenness (C), Total number of organisms (D)

Fungal Families in Cereal Rye

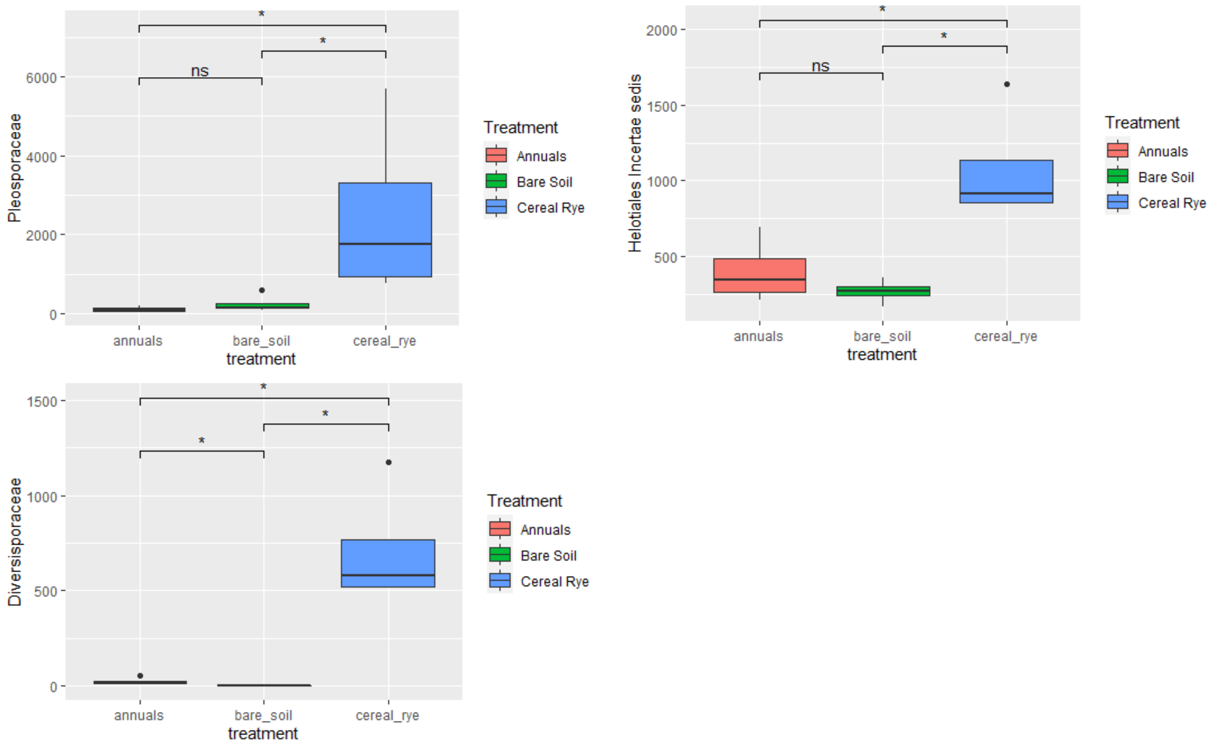


Figure 11. Fungal Families displaying higher number of organisms in cereal rye soils but not in annuals or bare soil.

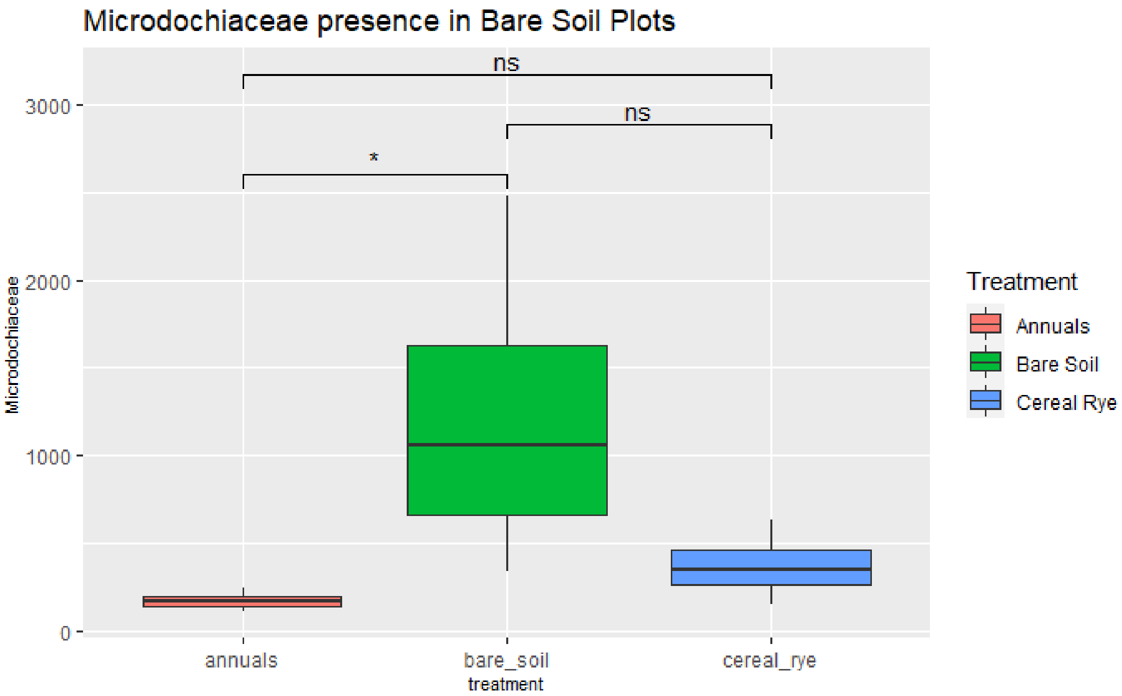


Figure 12. Significant difference in fungal family *Microdochiaceae* presence in bare soil.

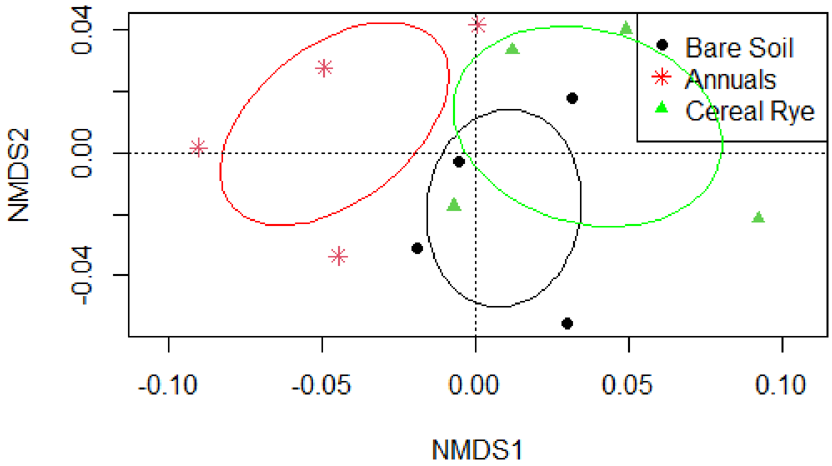


Figure 13. NMDS Ordination analysis of the relationship between bacterial families and treatments, ellipses represent standard deviations of point scores of each treatment.

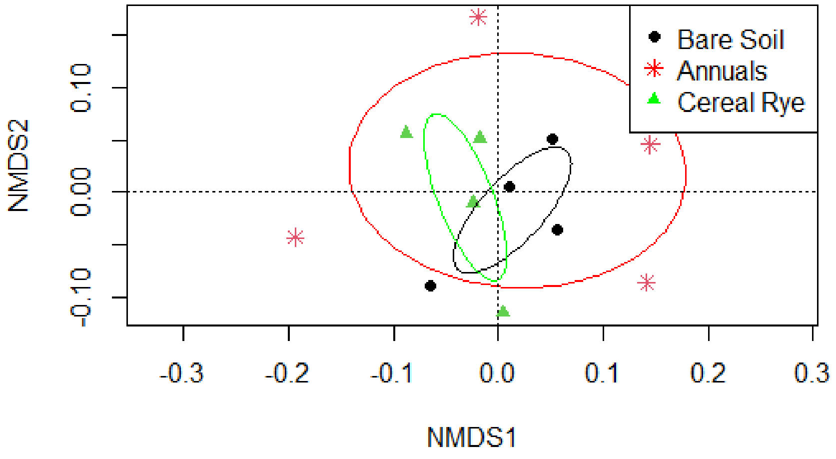


Figure 14. NMDS Ordination analysis of the relationship between fungal families and treatments, ellipses represent standard deviations of point scores of each treatment

Figure 15. Photo of annuals present in plots. Dead nettle (*Lamium purpureum*) and the common violet *(Viola sororia*) being the most abundant.

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CURRICULUM VITAE

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Research focuses on the ecological foundations of sustainable agriculture methods and how they can mitigate climate change. Aspire to use research to drive agricultural and climate policy change.

Education

Master of Science: Sustainability **2020-Current**

University Of Louisville - Louisville, KY

- Coursework in Environmental Policy, Ecology, Biostatistics, and Sustainable Systems
- Thesis: How do Cover Crops Change Soil Health in a No-Till System?

Bachelor of Arts: Biology -Ecology **1999-2003**

Bryn Mawr College - Bryn Mawr, PA

- Minor in Psychology
- Thesis: Juvenile and Adult Social Interactions in White Faced Capuchins

Research Experience

Master's research in soil microbe diversity (unpublished) **2021**

University of Louisville, Louisville, KY
In collaboration with Dr. Tamara Sluss and Dr Erin Haramoto

- Sampled soils, bulk density, and pH of no till agriculture with differing cover crops.
- Assessed ITS and 16S sequencing

Data Analysis for COVID in waterways (unpublished) **2021**

University of Louisville, Louisville, KY
In collaboration with Cullen Hunter and Dr. Tamara Sluss

- Analyzed data in R for publication (currently unpublished)

Research Assistant **2006 - 2008**

University of Kentucky, Lexington, KY

- Ran microbiology labs, PCR, gel electrophoresis, and cell culture.
- In charge of my own project on HIV cells.

Undergraduate Research (unpublished) **2002 - 2003**

Bryn Mawr College, Bryn Mawr, PA & La Suerte Biological Field Station, Costa Rica

- Observed interactions of *Cebus imitator* in the wild
- Presented for undergraduate defense

Related Work Experience

Regenerative Agriculture Initiatives Intern **Fall 2021**

American Farmland Trust, Louisville, KY

- Maintain spreadsheets and run data in R for climate change project and Rye Initiative.
- Ran data in R for analysis and presentation to farmers
- Collaborate with corresponding AFT regional offices with climate change project data.
- Network and engage with farmers, researchers, and end-users during AFT events.

President **2020-Present**

Shelby County Farmers Market, Shelbyville, KY

- Previous Board Member and Secretary
- Ran directive to meet standards for COVID requirements to ensure opening during the 2020 season
- Ran marketing, promotions, and community engagement efforts
- Developed recruiting and orientation tools for new vendors

Co-owner and Operator **2015-Present**

Moonlight Farms Homestead, Pleasureville, KY

- Plant and maintain garden beds using regenerative farming methods.
- Run community supported agriculture (CSA)
- Developed and maintained relationships with customers through CSA and Farmers Market.

Policy Groups

Community Farm Alliance: Climate Change Working Group
National Healthy Soils: Policy Network
National Sustainable Agriculture Coalition: Conservation, Energy, and Environment Committee
Climate Justice Alliance: Green New Deal/Energy Democracy Merge Group

Affiliations

Organic Association of Kentucky (OAK)
Henry County Beekeepers
Community Farm Alliance
HEAL Food Alliance
Kentucky Conservation Committee

Conferences Attended

Organic Association of Kentucky (OAK) Conference 2020
Organic Association of Kentucky (OAK) Conference 2021 (Virtual)
National Sustainable Agriculture Coalition: Summer Conference (Virtual) 2021