

Can Crop Drought Resilience Be Improved with Alternative Soil Management Methods Via Mycorrhizal Fungal Associations?

Lucy Bennett

ABSTRACT

Drought conditions are increasing under global warming, threatening crops and the sustainability of agriculture. Soil management methods have the capacity to improve crop drought resilience by maximizing the ecosystem services of plant-fungal interactions. In the rhizosphere, arbuscular mycorrhizal fungal (AMF) can provide beneficial services by their associations with crop roots improving water uptake and drought resilience. This research investigates the impact of tillage and cropping regimes on the drought resilience of the experimental crop, black bush beans, *Phaseolus vulgaris*, and its associated AMF community. The bean crops were grown under simulated drought conditions for a 3-month growing period. Plant, bean, root, and fungal samples were collected once the crops were mature. We found that plant biomass and pod production were significantly higher in the cover crop treatments, but the AMF colonization was significantly lower. In contrast, the continuous production treatment had lower plant biomass and pod production but higher AMF colonization. All of the plant and fungal metrics were slightly, but not significantly, higher in the no till treatments. These results suggest that AMF colonization in agroecosystems is not necessarily associated with higher crop yields and that field cropping regimes are significant factors. Further research is needed to better understand the potential costs and benefits of particular AMF taxa with specific crop varieties. The interaction of tillage, cropping regimes, and drought conditions in field experiments have the potential to inform soil management practices for sustainable farming under climate change.

KEYWORDS

Arbuscular mycorrhizal fungi, climate change, cover cropping, continuous production, ecological intensification, plant-fungal interactions, tillage

INTRODUCTION

Climate change is increasing the severity and frequency of drought worldwide, threatening agricultural production and our food systems. This is especially true in California, where scarce water resources make drought a significant threat to agricultural systems (Pathak et al. 2018). At the same time, conventional, industrialized agriculture has substantial energy and environmental costs for ecosystem functioning and even human health. Only about half of the nutrient and inorganic fertilizer inputs are taken up by crops (Robertson and Vitousek 2009) and this waste has consequences for biodiversity loss, run off, and climate change (Erisman et al. 2014). These challenges highlight why we need to replace reliance on ecologically harmful inputs and shift towards ecosystem intensification in agroecosystems.

Sustainable farming practices such as no till and cover cropping have been shown to improve soil health. No-till practices, which involve minimizing soil disturbance and retaining living root structures, have high levels of crop residue, increasing soil organic carbon and total soil nitrogen content (Omara et al. 2019). No till practices also increase the microbial- derived organic matter within water stable soil aggregates (Simpson et al. 2004). Cover crops are crops that are grown to benefit soil biology and functioning, rather than for human consumption, and have been shown to reduce erosion, enhance weed suppression, and improve nutrient retention (Schipanski, et al., 2014). Along with improving soil health, these practices improve crop health and drought resilience.

The benefits of these alternative soil management methods to crops may be partly attributed to the increased health and proliferation of soil organisms, such as symbiotic mycorrhizal fungi (Lehman et al. 2012, Schmidt et al. 2019). Arbuscular mycorrhizal fungi (AMF) is one of the most ubiquitous symbiotic fungi, forming associations with 80-90% of terrestrial plants and virtually all staple food crops (Smith & Read 2008). Reduced disturbance regimes, such as no till and cover cropping, can change AMF community composition (Jansa et al. 2003) and potentially enhance AMF community diversity (Oehl et al. 2009). In a review of field studies across five continents by Bowles et al. 2016, arbuscular mycorrhizal fungal colonization of cash crop roots was shown to be positively affected by cover cropping and alternative tillage practices. The extent of AMF colonization in crop roots is dependent on the history of soil management of the site as well as crop type and even the AMF taxa (Hart and Reader 2001).

The potential benefits of AMF as a component of sustainable agriculture can come from their functioning in individual plants as well as in the wider agroecosystem. In crops, AMF mediate nutrient acquisition by increasing effective root surface area and solubilizing crop nutrients (Hooker and Black 1994), in particular nitrogen (N) and phosphorus (P), and can diversify sources of N for crops (Thirkell, Cameron and Hodge 2016). AMF can increase host plant biomass and enhance yields (Lehman et al. 2012), improve defenses to herbivores and pathogens (Shrivastava et al. 2017, Thirkell et al. 2017) and tolerance to heavy metals and salinity (Kumar et al. 2015). Soil with abundant AMF have improved soil structure and increased aggregates from the extra-radicle mycelia (in the soil) (Rillig & Mummey 2006) which can provide ecosystem services such as increased soil organic matter from AMF secretions (Morris et al. 2019) and increased soil carbon sequestration (Verbruggen et al. 2016). AMF can also improve host crop drought tolerance (Augé 2004) through several different means. AMF mycelium in crop roots can increase water uptake in plants (Cavagnaro 2016), alter the leaf water potential (Porcel and Ruiz-Lozano 2004) and change stomatal conductance to adapt to drought conditions (Augé 2015). AMF in soils also improve soil water holding capacity (Rillig & Mummey 2006) and tightly control water loss (Lazcano et al. 2014).

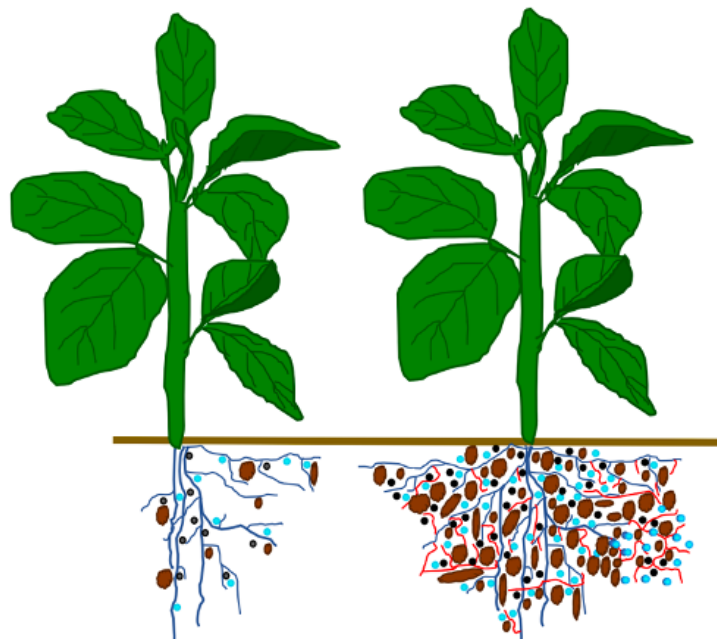


Figure 1. AMF supported water retention. From Thirkell et al., 2017: “Rhizosphere soil in proximity to a plant [roots in dark blue] colonized by AMF [in red] (right), may show higher water retention (blue), organic carbon input and sequestration (black), as well as greater soil aggregate stability (brown).”

Although the benefits of AMF are extensive, there are limitations to AMF that are context dependent. The ‘trade-balance’ model suggests that if soil nutrients, such as N and P, are limiting, then AMF colonization would benefit host plant growth (Johnson 2010). This is why in arable and fertilized soils, where N and P are not limiting nutrients, crops are generally unresponsive to AMF inoculation, which has been shown for cereal crops in soils with abundant P (Li et al. 2005). Further, soil management methods that heavily apply chemical fertilizers and that disturb the soil with tillage, have the capacity to shift mycorrhizal associations from a beneficial to a neutral relationship (Jansa et al. 2003) or even a parasitic relationship with the plant hosts (Johnson et al. 2015). Plant acquisition of nutrients from a common mycelia network is more dependent on plant identity rather than determined by the supply of carbon from the plant to the network (the ‘reciprocal rewards’ model) (Walder et al. 2012). In general, AMF seems to be most beneficial to the host plants when the soil management regimes are the least disruptive to the soil and soil nutrients are limiting, however, that relationship depends on the plant variety and AMF taxa. Therefore, the benefits of AMF in agroecosystems cannot be predicted and are dependent on context.

In this thesis, we established four soil management treatments, and examined their impact on AMF colonization of bean crops, and the subsequent performance of those crops in drought conditions. The four soil management treatments included conventional tillage and no till in combination with continuous production and cover cropping regimes. The performance of the crops, black bush beans, *Phaseolus vulgaris*, was quantified by measuring plant biomass, shoot biomass, bean pod counts and bean mass. Stem water potential was measured, as a metric of plant drought stress, as well as soil water content. AMF colonization was quantified by microscopy methods that measured the extent of mycorrhizae embedded in the crop roots. We discuss the implications of our results for soil management as they related to AMF colonization and drought tolerance in their host crops in an agroecology setting.

METHODS

Experimental Setup

Our study was conducted on the Oxford Tract Research Station at UC Berkeley, on a long-term no-till and cover cropping experiment in a factorial, randomized block design. In June, 2019 we planted a variety of black turtle bush beans (*Phaseolus vulgaris*) from Peaceful Valley. We chose black beans for the study because they readily form associations with AMF and because they are of interest to farmers since they fix nitrogen and are a staple food crop.

The factorial combination of tillage and cropping regimes yielded four distinct soil management systems. Our no-till management system was designed to minimize soil disturbance and maintain living root structures, with ample application of compost to the soil surface. The control was a conventional till method that included ripping and reforming the soil with a tractor-driven disk harrow. On top of this, we overlaid two cropping systems. The first was a continuous production system where broccoli, *Brassica oleracea* var. *Italica*, was grown in the winter before the summer bean crop. The broccoli heads were harvested in winter (hence continuous production) and their roots were left behind in the soil. The second cropping regime utilized a daikon radish cover crop, *Raphanus sativus* var. *Longipinnatus*, grown in the winter before the summer bean crop. Daikon radish have large taproots that were left to decompose in the soil, and have been shown to improve water retention and reduce compaction (Chen and Weil 2010, Gruver et al. 2016). Both of these crops are in the same plant family, Brassicaceae, which do not form associations with AMF. Prior to our experiment, these tillage and cropping treatments had been employed for two years.

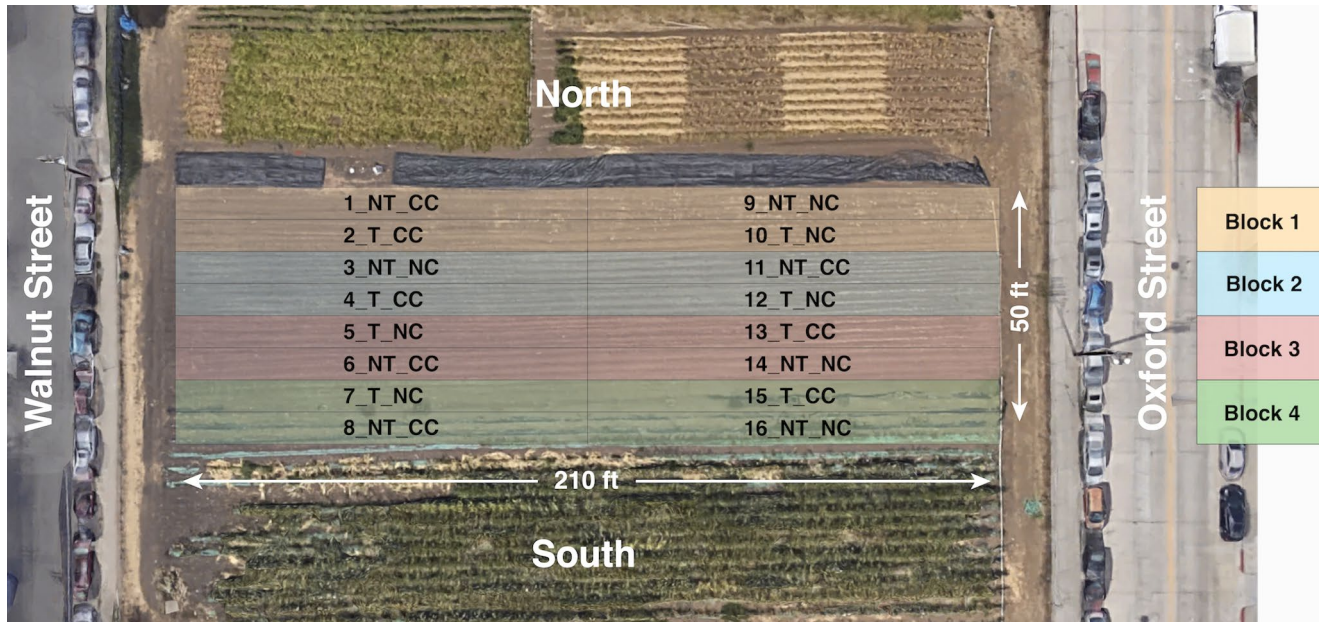


Figure 2. Above image of the Oxford Tract Research Field. The soil management treatments on each plot, on the Oxford Tract at UC Berkeley. The treatments are labeled; T: tillage, NT: no tillage, CC: cover crop, CP: continuous production, and the four blocks (shown on the right) are labeled 1- 4.

The combination of these factors makes four treatments, which were replicated four times for a total of 16 plots (Figure 2). The plots were arranged in a split plot design where tillage was the main factor and was applied per row (two adjacent plots) as dictated by the tractor. The cropping regime factor was the split factor (applied per plot). Four experimental blocks were created that contained a complete set of the four soil management treatments, numbered 1-4 (see right of figure). The field was also subject to a drought simulation. For the first month of the crop's growth, the irrigation level was set to replace 100% water lost from evapotranspiration. Evapotranspiration (ET) was measured using the CIMIS database and using relevant parameters for our particular soil and model crop. After germination, field irrigation was set to 50% ET, and after flowering, irrigation was cut off completely at 0% ET.

Plant Performance and Soil Metrics

Plant biomass and total bean yield

Plant biomass and bean yield were measured at the time of harvest. The total amount of above-ground plant biomass was determined from a 1m section of each plot and recorded as both the total

biomass in that meter as well as normalized to biomass per plant. The biomass was also desiccated, and the dry biomass was measured. We also selected four random plants from each plot to weigh the fresh shoot and dry shoot mass as well as the fresh and dry mass of the pods from those plants. The total number of bean pods from each plot were counted. The bean yield was measured by separating, or “threshing” the beans from the pods and the other plant material and weighing the total beans harvested from each plot.

Stem Water Potential

Stem water potential was measured five times from plants in each of the 16 plots during the drought stress test. The stem water potential was determined by covering a leaf with a bag for 15 minutes (so that the stomata close) then cutting that leaf off of the plant. Then that leaf was placed in a Model 615 Pressure Chamber Instrument, tightening the opening to the chamber around the stem of the leaf and pressurizing the chamber until the stem and chamber reached equilibrium (Meron et al. 1987). That equilibrium vapor pressure within the stem is defined as the stem water potential. Stem water potential measures the difference between the water pressures in the plant compared to the soil, and it becomes more negative as the soil dries, making this a proxy for the water stress the plant is experiencing.

Soil Moisture Content

Soil cores were taken when the beans were harvested from 0-5 cm, 5-15 cm, 15-30 cm, and 30-50 cm to evaluate soil moisture content. Soils were sieved to 2 mm, weighed out to 40 g, and then oven dried at 105 C for 24 hours. The samples were then weighed again, and the difference in mass was calculated as the total soil moisture for that sample.

AMF Colonization

Root Collection

Root samples from the black bean plants were taken for AMF colonization measurements by digging up a cylindrical section of soil about 1” away from the main stem of the plant with a trowel, then pulling out live roots out. Three plants were sampled and combined into one sample. The roots were cleaned with water to remove the excess soil and then fixed in ethanol. One sample was taken from each of the 16 plots at the end of the drought stress test, just before the plants were harvested.

Root staining and microscopy

The bean roots were fixed in ethanol in 15 mL Fischer vials and then the mycorrhizal colonisation of the roots was visualised using the staining technique of (Brundrett et al. 1994). The bean roots were cleared in 10% (w/v) KOH (80°C, 20 min), then rinsed in distilled water and stained in a 5% vinegar in Trypan Blue ink solution (20 min). The stained roots were mounted on a microscope slide, choosing a subset of 5 roots from each sample, making one slide per sample from that soil treatment plot, resulting in a total of 16 slides. The presence or absence of fungal hyphae, vesicles, and arbuscules were recorded using the gridline intersect method (McGonigle et al. 1990). On each slide, 100 intersections were observed resulting in a percentage of the extent of fungal colonization.

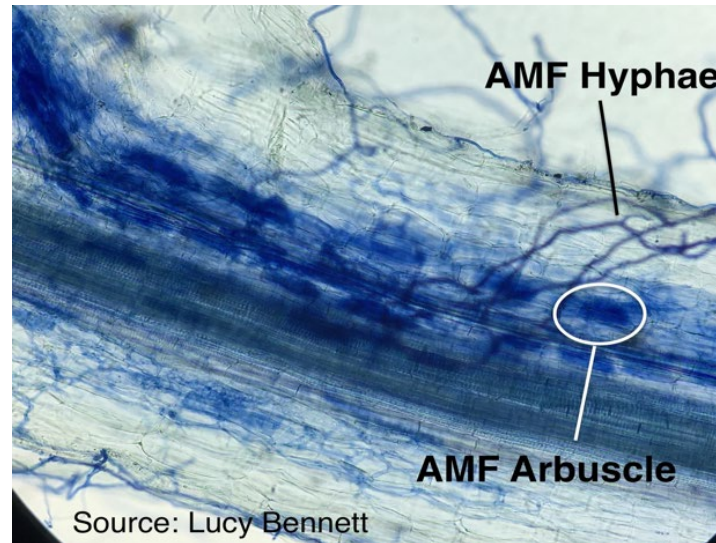


Figure 3. Image of AMF hyphae and arbuscules. This is an image of a cross section of a bean root colonized by AMF with visible hyphae and arbuscules under microscope (100X).

Statistical analysis

We used a linear mixed effects model in R to look for significant differences in plant performance, soil water content, and AMF colonization variables across the four soil management treatments (NT_CC, NT_CP, T_CC, T_CP) (R Development Core Team 2014). This model took into account variation between the blocks (describing location on the field, as seen in figure 2) considered a “fixed” effect in our regression analysis. We then used ANOVA to determine the significance between the treatments for each of our variables.

RESULTS

The cropping regime had a significant impact in both plant performance metrics, soil water content and AMF colonization. Neither tillage regime, nor its interaction with cropping regime, had a significant impact on any of these response variables. The cover cropped treatments had improved plant performance, and soil water content, compared with continuous production, during the imposed drought conditions. AMF colonization, on the other hand, was highest in the continuous production treatments. All of the values are in table 1 in the appendix.

Plant Performance

Fresh plant biomass normalized per plant in 1m was significantly higher ($F = 5.66$, $p < 0.05$) in the cover crop (daikon radish) and no till treatments, the average mass was 0.43 kg, and the max was at 0.71 kg. Dry plant biomass (per plant in 1m) was also higher in the cover crop no till treatment. The cropping regime was the significant factor, but not the tillage regime nor the interaction between cropping and tillage.

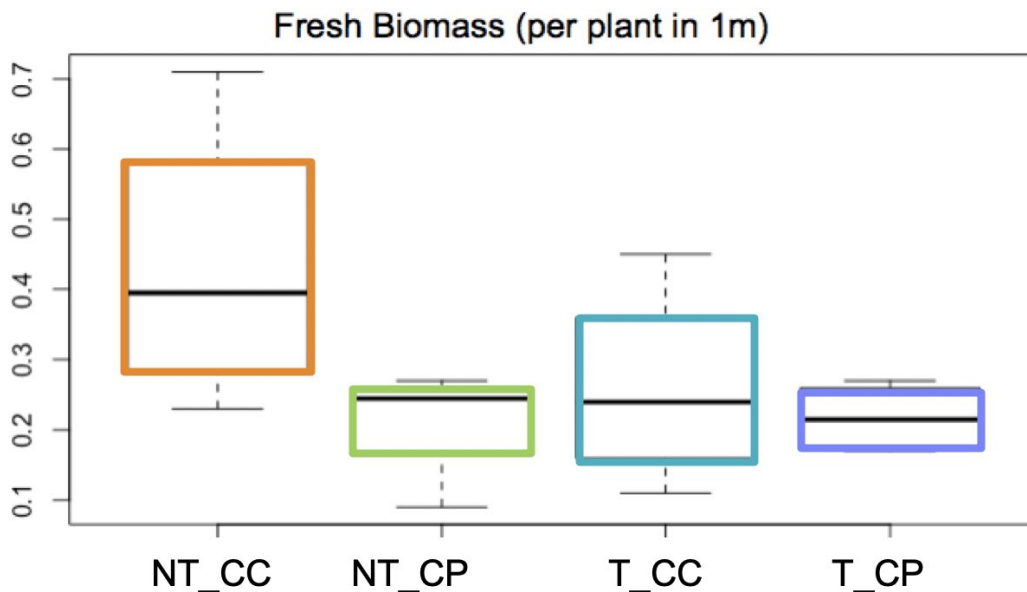


Figure 4. Fresh biomass (g) normalized per plant in 1 meter. The fresh biomass per plant in 1m was highest in the cover crop no till treatment (NT_CC, in green) and significantly higher in the cover crop treatments.

The greatest shoot biomass per four plants was also in the cover cropped (daikon radish) no till treatments. The cropping regime again was the significant factor for shoots as measured in fresh shoots ($F = 5.76$, $p < 0.05$), and for dry shoots ($F = 4.23$, $p < 0.10$).

The mass of the fresh pods, from those plants in the 1m section, was significantly higher in the cover crop treatments ($F = 5.40$, $p < 0.05$) and higher in the no till. The total number of bean pods per plot was also significantly higher in the cover crop treatments ($F = 5.22$, $p < 0.05$) and higher in the no till. The total bean yield, measured as the weight of the beans separated from the pods, from each plot, was not significantly different between treatments (see appendix 1).

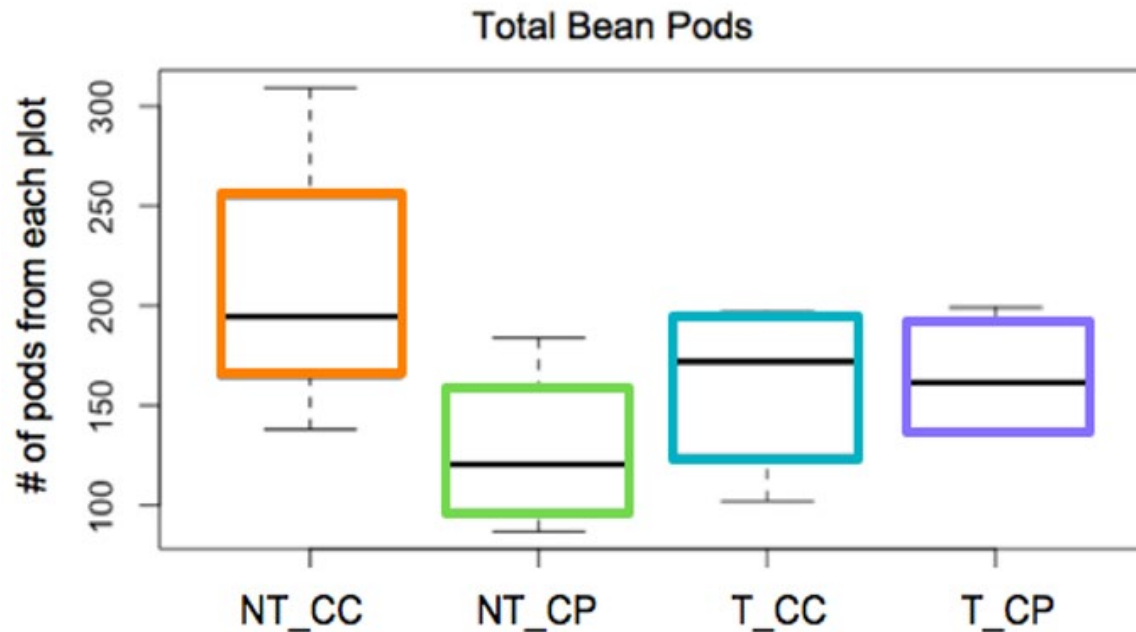


Figure 5. The total bean pod production. The total bean pod count per plot was highest in the cover crop no till treatment (NT_CC, in orange) and significantly higher in the cover crop treatments.

Plant water stress and soil water metrics

The stem water potential data was not significantly different between the treatments. However, over the 5 time points, it did consistently increase, showing that the plants were becoming more water stressed throughout the drought stress test.

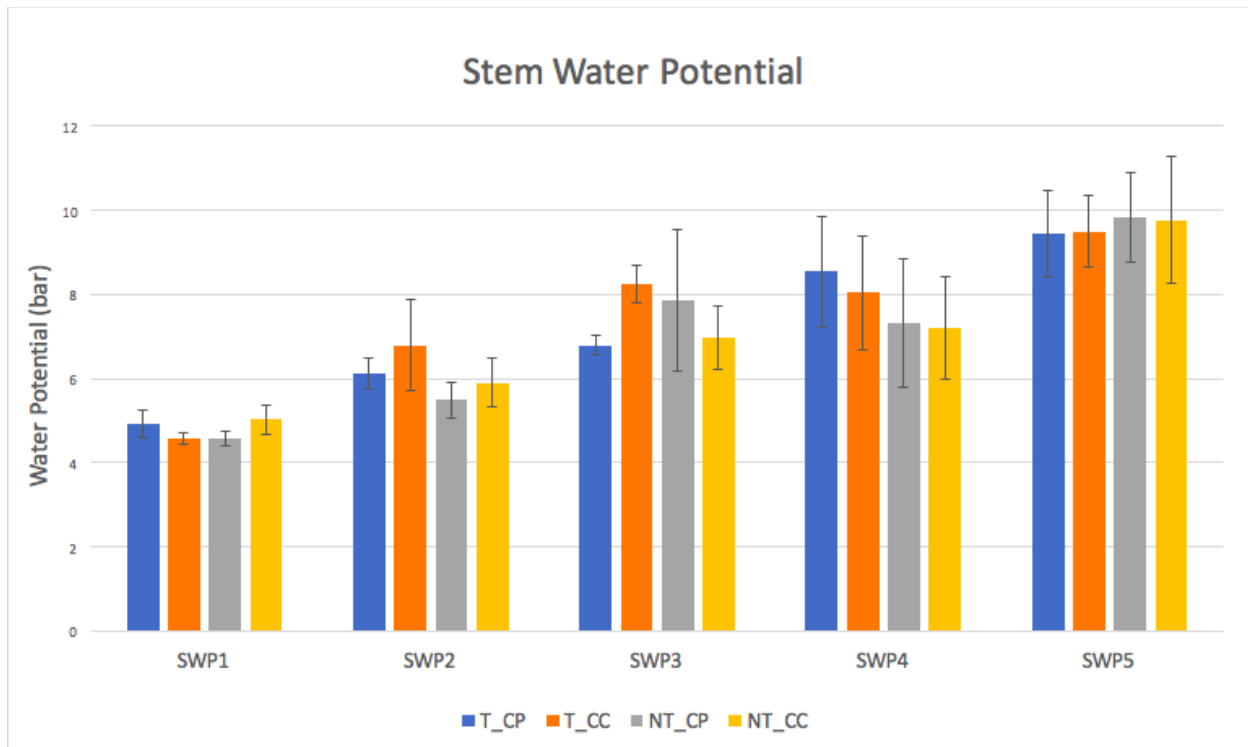


Figure 6. Stem water potential across all soil management treatments. The stem water potential was not significantly different between the four treatments, and, in all treatments, it increased from week 1 to week 5 as the drought stress increased, the histogram shows the average value with standard deviation.

The soil water content at the end of the drought simulation was significantly higher in the no till treatment at depth 0-5 cm but then significantly higher in the cover crop treatments at depths 15-30 cm, and 30-50 cm (see appendix 1).

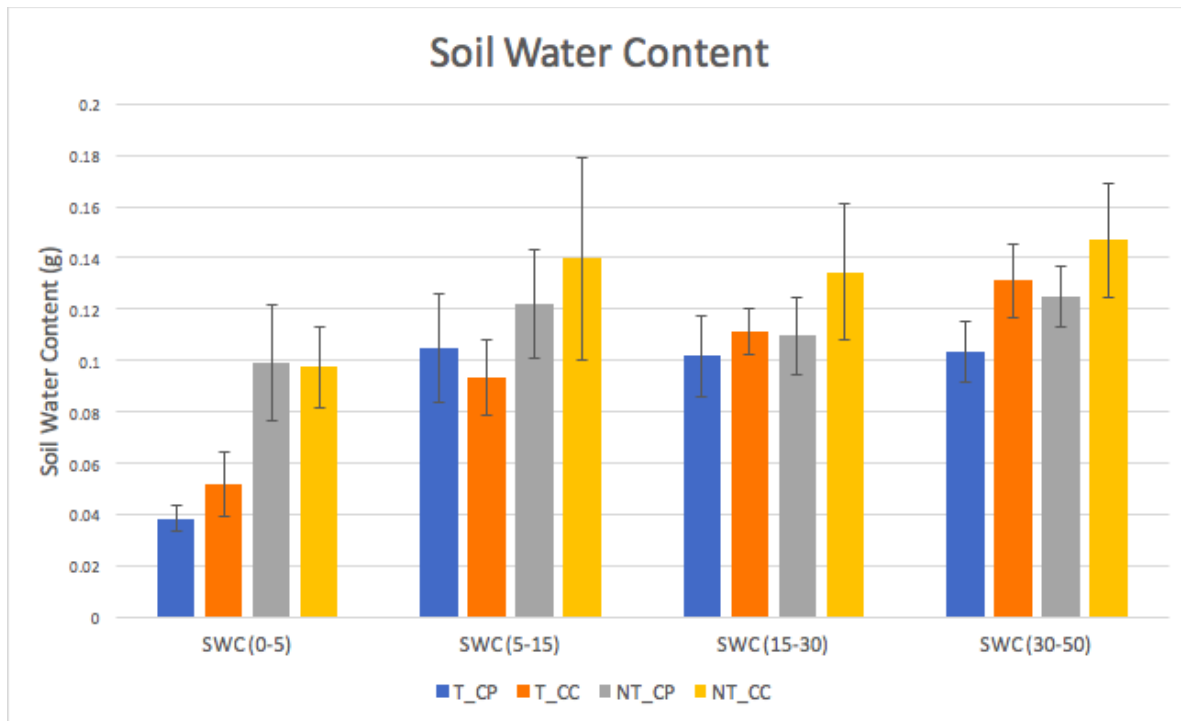


Figure 7. Soil water content in all treatments at different depths. The soil water content was highest in the no till and cover crop treatments at depths 15 through 50 cm, the histogram shows the average value with standard deviation.

Field Conditions (Blocks)

Our linear mixed effects model took into account the variations between the blocks (see figure 2) on our field. The variation between the blocks was significant, with p values ranging from 0.05 to 0.001, for the fresh and dry biomass (total and per plant), fresh and dry shoots, total pods, stem water potential at each timepoint, soil water content at all depths, and AMF colonization (total structures). This was an indication that conditions differed significantly across the field, from north to south. The southern block (4) is on the downward side of a slope and is where we observed more water, higher organic content, and better plant performance.

AMF Colonization

The total AMF colonization (the sum of all the structures observed) was significantly highest in the continuous production ($F = 5.66$, $p < 0.05$) and no till plots, with an average of colonization in those plots. The fungal hyphal counts were also to be significantly higher in the continuous production plots ($F = 7.11$, $p < 0.05$). Vesicles were highest in the continuous production and no

till treatments, but arbuscules (which are unique to AMF) were highest in the cover crop and no till treatments. However, neither vesicle nor arbuscule counts were shown to be significant (see appendix 1).

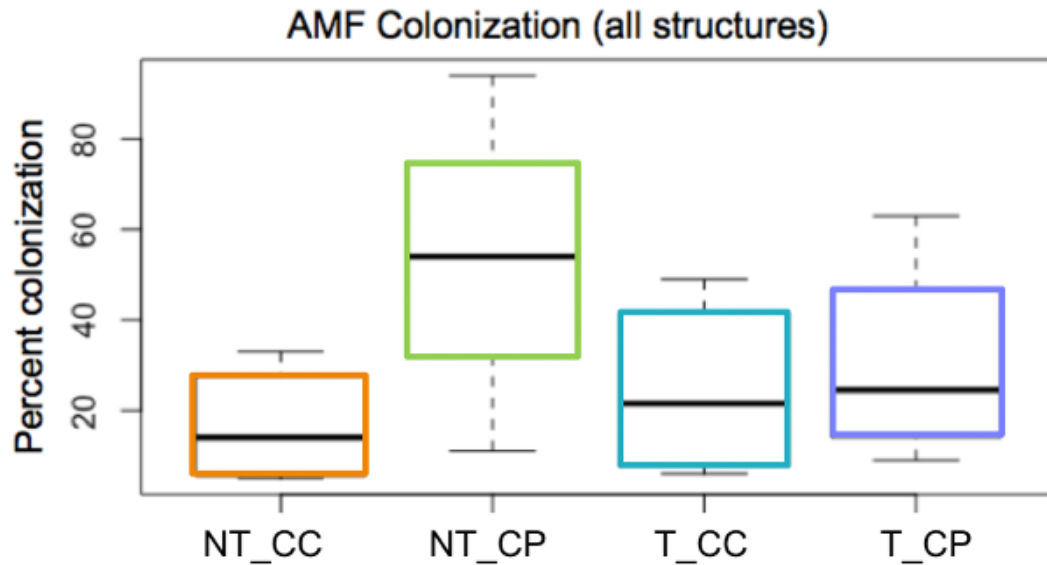


Figure 7. AMF colonization percentages for all structures. (sum of the hyphae, vesicle, and arbuscule counts) was highest (green boxplot) in the no till and continuous production plots.

DISCUSSION

Cover crops and crop drought resilience

One of our main findings was that crop performance (measured as plant biomass and bean pod total and weight) and soil moisture content was significantly higher in the cover crop treatments. This result was also in the context of the imposed drought stress, and so it is evidence that the cover crop increased the bean crop drought resilience. These findings are further support for soil management practices where cover crops are used to not only improve soil health and structure (Schipanski, et al., 2014), but subsequently, crop drought resilience.

In our study, we used a daikon radish cover crop, which was chosen because, as it decomposes, organic matter is incorporated deep into the soil (Gruvur et al., 2016). Daikon was also used because, in the no till soil management system, the radish functions as “biological till,” meaning it gradually breaks apart the hardpan, which can develop without tillage, as the large

taproot becomes established (Chen & Weil, 2010). These fissures in the soil from the taproot improve infiltration, surface drainage, improving soil porosity and subsoil moisture, resulting in improved drought resilience (Chen & Weil, 2010). Further, as the cover crop increases available soil nutrients, this results in larger plants (as our results found) which can better withstand drought conditions.

Stem water potential and total bean mass were both not significantly different between the management treatments, and the small sample size could have impacted the significance of those two variables.

Spatial variation

We found that the variation across the field, divided into blocks (a block included all four treatments, see figure 2), was also significant ($p < 0.001$). This means that the spatial variation across the field, north to south, was also significant, in addition to the variation between our soil management treatments. One possible reason for this variation is that the Oxford Tract research field has a north to south slope and, from our observations, the southern part, which is lower, collects more water and has higher organic matter in the soil. However, there may be other factors causing the heterogeneity across the field. This demonstrates how variations across small agricultural fields are magnified and leave significant impacts. This is especially relevant for small farmers and urban farmers. Urban farmers are most subject to variation in their fields as they, most often, have small plots of land and because the past history of urban soil is incredibly variable and carries lasting effects.

Tillage factor

The highest counts for all of our response variables were in the no till treatments, however, tillage was never a significant factor. This may be, in part, because our experiment had few replicates ($N=4$), and perhaps with more replicates, tillage would be significant. It may also be because the Oxford Tract has been under this tillage regime for two years before the experiment and the impacts of no till takes many years to be fully realized. However, tillage is known to disrupt mycelium (Jansa et al., 2003; Kabir, 2005; Bowles et al., 2016) which makes the absence of

significant effects from tillage on the AMF colonization data particularly surprising. Tillage disturbance, however, can instead result in a shift in the fungal community by favoring more disturbance resilient taxa (Oehl et al., 2009; Schmidt et al., 2019) and so would not necessarily decrease colonization counts. Colonization is only one measure, and a limited measure, of the fungal community.

AMF colonization

Our results found that AMF colonization was significantly higher in the continuous production treatments, whereas plant performance, and soil water content, were both significantly lower. In the continuous production system, broccoli was grown in the season before the black beans, and they are known to be “heavy feeders” on soil nutrients. AMF are shown to be most beneficial to plants in soils with limited nutrients (Thirkell et al. 2017) and so the crops in the continuous production plots would benefit most from colonization. AMF have also been shown to increase crop drought tolerance (Augé 2004, Augé et al. 2014, Srivastava et al., 2017). These potential benefits, however, do not seem to be supported by the reduced plant performance seen in the plots continuous production plots. Also, in many of the studies of these benefits of AMF, the researchers inoculated their experiment with AMF. In this work, we did not inoculate the field and instead, our colonization counts were a measure of the (visible) fungus in the roots that originated in the soil from the field.

Fungal taxa

Fungal DNA samples were taken from both the bean roots and from soil in the rhizosphere, and the ITS2 region was amplified and sequenced. The analysis of these samples is in progress; however, our preliminary findings are that in the root and soil fungal communities, Glomeromycota (AMF) are just one of many phyla observed (Rainey and Bennett, unpub. data). Therefore, the colonization data likely include some colonization by other fungal taxa, which may have different functions, and relations to the host, than AMF. For instance, one of the identifiable guilds from this taxonomic data are plant pathogens. If the pathogenic fungal presence was significant in the roots, that might explain the lower plant performance in the treatments with

higher fungal colonization. The plants in the continuous production treatments would be more susceptible to fungal pathogens (Huber et al. 2012) if the soil was depleted by the treatment. These taxonomic data, once analyzed, will inform our understanding of the role of fungi in this experiment.

Future directions

A limitation of root colonization measurements is that they do not indicate the function of the fungi, which would be addressed by sequencing the fungal DNA. The next step for this research is to complete the analysis of the fungal taxonomic data. We will determine the relative OTU abundance, using a Shannon-Weaver Index to measure diversity, as well as measuring community richness, community structure, composition, and analyzing indicator species. There are also limitations to the DNA analysis of the fungal OTU's. For instance, the sequencing is not able to identify all taxa, or determine the function of all taxa, especially as function changes under different conditions. Also, DNA sampling and extraction is also limited and so does not perfectly reflect the DNA in the environment. These DNA data, however, will be very helpful for our understanding of the fungal community as well as its potential functions in this experiment.

Our data showed that fungal colonization, from AMF and other fungi, was significantly higher in the continuous production treatments. However, there are limitations to “colonization” as a measure of the extent of the fungal community. Colonization measurements are not normalized per plant, it does not include soil fungi (which affect soil water holding capacity), nor does it account for fungi that cannot be visualized (do not take up the blue stain). Future research could address some of these limitations by including root biomass measurements, in order to normalize the colonization by root biomass, and soil hyphal length, to quantify the extent of the soil fungal community.

Future research should continue to look at experiments that are conducted in the field and that follow crops from seed to harvest as this type of study is under-represented in the body of AMF literature. Our work showed the importance of the winter cover crop in the success of the beans as well as the extent of the fungal associations. Therefore, we have found that AMF (and other fungal) functions are dependent on the soil management history, and it is important for research to be conducted on land such as the Oxford Tract, where there is a known site history.

Field experiments using sustainable soil management practices will help inform the science supporting drought resilience, soil health and soil management practices in an ever-drier climate.

Broader impacts

California is facing increasingly severe consequences to agricultural systems from climate change including scarce water resources (Pathak et al. 2018). With an improved understanding of the below-ground interactions that aid in crop drought resilience, we could adapt and protect our food system. Shifting towards soil management practices that maximize the ecosystem services of mycorrhizae may be a part of agriculture's adaptation. Our research explored soil management factors of urban agricultural soil that influence mycorrhizae and drought resilience, and this can inform the practices of farmers facing water scarcity, in particular small farmers and urban farmers.

ACKNOWLEDGEMENTS

I would like to first thank Coleman Rainey, my mentor, who has helped me every step of the way on this project, answering so many of my questions and learning along with me as we explored this topic. He is currently working on his PhD in the Bowles lab and he is a steward of the Oxford Tract field experiments. I would also like to thank Professor Timothy Bowles, who oversaw this work and whose own research taught me so much. More thanks to Arely Ortiz who taught me everything I know about identifying AMF under the microscope, and Aidee Guzman who taught me all about the fungal DNA analysis and PCR workflow, and to the rest of the Agroecology lab. I would also like to thank the whole ESPM 175 team and class for their guidance, support, and the structure that a class provides. I would like to acknowledge and thank the Sponsored Projects for Undergraduate Research (SPUR) office for the Student-Initiated Research Grant which funded the fungal DNA analysis. Lastly, I would like to thank all the people involved with Defend the Oxford Tract for fighting for the right to grow food on the University's land, originally Ohlone land. Also, thanks to the Student Food Pantry in UC Berkeley's Basic Needs Center, which distributes the food we grow on the Oxford Tract directly to students and other folks in need.

REFERENCES

- Augé, R. M. 2004. Arbuscular Mycorrhizae and Soil / Plant Water Relations. *Can. J. Soil Sci.* 84: 373–381
- Augé, R. M., H.D. Toler and A.M. Saxton. 2015. Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. *Mycorrhiza*, 25, 13–24.
- Brundrett, M., L. Peterson, L. Melville, H. Addy, T. McGonigle, G. Schaffer. 1994. *Practical Methods in Mycorrhizal Research*. Toronto ON: Mycologue Publications.
- Bowles, T. M., et al. 2016. Ecological Intensification and Arbuscular Mycorrhizas: A Meta-Analysis of Tillage and Cover Crop Effects. *Journal of Applied Ecology*, vol. 54, no. 6 pp. 1785–93, doi:10.1111/1365-2664.12815.
- Cavagnaro, T. R. 2016. Soil moisture legacy effects: impacts on soil nutrients, plants and mycorrhizal responsiveness. *Soil Biology & Biochemistry*, 95, 173–179.

- Chen, G., and R. R. Weil. 2010. Penetration of cover crop roots through compacted soils. *Plant and Soil* 331: 31–43. (Available online at: <http://dx.doi.org/10.1007/s11104-009-0223-7> <http://dx.doi.org/10.1007/s11104-009-0223-7>)
- Erismann, J. W., Galloway, J. N., Seitzinger, S., Bleeker, A., Dise, N. B., Petrescu, R., Leach, A. and de Vries, W. 2014. Consequences of human modification of the global nitrogen cycle. *Philosophical Transactions of the Royal Society B*, 368, 1–9.
- Gruver, J., Weil, R. R., White, C., Lawley, Y. 2016. Radishes - A New Cover Crop for Organic Farming Systems. *Organic Agriculture*. 20160226.
- Hart, M. M. and R. J. Reader. 2001. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist*, 153: 335-344.
- Hooker, J. E., and K. E. Black. 1995. Arbuscular Mycorrhizal Fungi as Components of Sustainable Soil-Plant Systems. *Critical Reviews in Biotechnology*, vol. 15: 201–12. doi:10.3109/07388559509147408.
- Huber, Römheld, Weinmann. 2012. Ch 10: Relationship between nutrition, plant diseases and pests. 283-297. Marschner. P. Marschner's Mineral Nutrition of Higher plants. El Sevier Science and Technology. London, UK.
- Jansa, J., Mozafar, A., Kuhn, G. and Anken, T. 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications*. 13: 1164–1176.
- Johnson, N.C. 2010. Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytologist*. 185: 631–647.
- Johnson, N.C., Wilson, G.W.T., Wilson, J.A., Miller, R.M. & Bowker, M.A. 2015. Mycorrhizal phenotypes and the Law of the Minimum. *New Phytologist*. 205: 1473–1484.
- Kabir, Z. 2005. Tillage or no-tillage: impact on mycorrhizae. *Canadian Journal of Plant Science*, 85: 23–29.
- Kaur R, Singh A, Kang JS 2014. Influence of different types of mycorrhizal fungi on crop productivity. *Curr Agric Res* 2:51–54.
- Kiers, E.T., Duhamel, M., Beesetty, Y. et al. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science*, 333, 880–882.
- Lazcano, C., Barrios-Masias, F.H. & Jackson, L.E. (2014) Arbuscular mycorrhizal effects on plant water relations and soil greenhouse gas emissions under changing moisture regimes. *Soil Biology & Biochemistry*, 74, 184– 192.
- Lehman, R.M., Taheri, W.I., Osborne, S.L., Buyer, J.S. & Douds, D.D. 2012. Fall cover cropping can increase arbuscular mycorrhizae in soils supporting intensive agricultural production. *Applied Soil Ecology*, 61, 300–304.

- Lekberg, Y. & Koide, R.T. 2005. Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. *New Phytologist*, 168, 189–204.
- Li, H.Y., Zhu, Y.G., Marschner, P., Smith, F.A. & Smith, S.E. 2005. Wheat responses to arbuscular mycorrhizal fungi in a highly calcareous soil differ from those of clover, and change with plant development and P supply. *Plant and Soil*, 277, 221–232.
- Meron, M., Grimes, D.W., Phene, C.J. et al. 1987. Pressure chamber procedures for leaf water potential measurements of cotton *Irrig Sci* 8: 215. <https://doi.org/10.1007/BF00259382>
- Morris, E. K., et al. 2019. Visualizing the Dynamics of Soil Aggregation as Affected by Arbuscular Mycorrhizal Fungi. *The ISME Journal*, vol. 13: 1639–46 doi:10.1038/s41396-019-0369-0
- McGonigle, T., Miller, M., Evans, D., Fairchild, G., and Swan, J. 1990. A new method which gives an objective measure of colonization of roots. *New Phytol.* 115, 495–501. doi: 10.1111/j.1469-8137.1990.tb00476.x
- Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Wiemken, A. & Boller, T. 2009. Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. *Agriculture, Ecosystems and Environment*, 134, 257–268.
- Omara, Peter, Lawrence Aula, Elizabeth M. Eickhoff, Jagmandeep S. Dhillon, Tyler Lynch, Gwendolyn B. Wehmeyer, and William Raun. 2019. Influence of No-Tillage on Soil Organic Carbon, Total Soil Nitrogen, and Winter Wheat (*Triticum Aestivum* L.) Grain Yield. *International Journal of Agronomy*, 1–9. <https://doi.org/10.1155/2019/9632969>.
- Pathak, Tapan B., et al. 2018. Climate Change Trends and Impacts on California Agriculture: A Detailed Review. *Agronomy*, vol. 8: 1–27, doi:10.3390/agronomy8030025.
- Porcel R, Ruiz-Lozano J. M. 2004. Arbuscular mycorrhizal influence on leaf water potential, solute accumulation and oxidative stress in soybean plants subjected to drought stress. *JExp Bot* 55:1743–1750
- R Development Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Rillig, M.C. and Mummey, D.L. 2006. Mycorrhizas and soil structure. *New Phytologist*, 171, 41–53.
- Robertson, G.P. and Vitousek, P.M. 2009. Nitrogen in agriculture: balancing the cost of an essential resource. *Annual Review of Environment and Resources*, 34, 97–125
- Schipanski, M.E., Barbercheck, M., Douglas, M.R. et al. 2014. A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agricultural Systems*, 125,12–22

- Schmidt, R. Mitchell, J. Scow, K. 2019. Cover cropping and no-till increase diversity and symbiotroph:saprotroph ratios of soil fungal communities. *Soil Biology and Biochemistry*, 129, 99-109, <https://doi.org/10.1016/j.soilbio.2018.11.010>.
- Simpson, Rodney T., et al. 2004. Preferential Accumulation of Microbial Carbon in Aggregate Structures of No-Tillage Soils. *Soil Science Society of America Journal*, vol. 68: 1249–55, doi:10.2136/sssaj2004.1249.
- Smith, S.E. and Read, D.J. 2008. *Mycorrhizal Symbiosis*, 3rd edn. Academic Press, Cambridge, UK.
- Srivastava, P., Saxena, B., Giri, B. 2017. Chapter 20: Arbuscular Mycorrhizal Fungi: Green Approach/ Technology for Sustainable Agriculture and Environment. Springer Int. Publishing AG 2017.
- Thirkell, T.J., Cameron, D.D. & Hodge, A. 2016. Resolving the ‘nitrogen paradox’ of arbuscular mycorrhizas: fertilization with organic matter brings considerable benefits for plant nutrition and growth. *Plant, Cell & Environment*, 39, 1683–1690
- Thirkell, Thomas J., et al. 2017. Are Mycorrhizal Fungi Our Sustainable Saviours? Considerations for Achieving Food Security. *Journal of Ecology*, vol. 105: 921–29, doi:10.1111/1365-2745.12788.
- Vierheilig, H., Schweiger, P., & Brundrett, M. 2005. An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiologia Plantarum*, 125(4), 393–404. <https://doi.org/10.1111/j.1399-3054.2005.00564>.
- Walder, F. & van der Heijden, M.G.A. 2015. Regulation of resource exchange in the arbuscular mycorrhizal symbiosis. *Nature Plants*, 1,7.

APPENDIX

Table 1. All response variables per plot with significance. The treatments are labeled; NT CC: no till cover crop, NT CP: no till continuous production, T CC: till cover crop, T CP: till continuous production.

Category	Variable (units)	NT CC		NT CP		T CC		T CP		Significant Factor	p Value
		Average	Std Dev	Average	Std Dev	Average	Std Dev	Average	Std Dev		
Plant Biomass	Fresh biomass, total in 1m (kg)	2.46	0.566	2.45	0.353	2.45	0.353	2.39	0.293	N. S.	N. S.
	Fresh biomass, per plant (kg)	0.328	0.267	0.260	0.206	0.260	0.206	0.218	0.050	Cropping	0.041
	Dry biomass, total in 1m (kg)	0.680	0.040	0.685	0.060	0.685	0.060	0.675	0.038	N. S.	N. S.
	Dry biomass, per plant (kg)	0.088	0.042	0.073	0.037	0.073	0.037	0.093	0.010	Cropping, Till:Cover	0.019, 0.014
Shoot mass	Fresh shoots (kg)	0.600	0.520	0.425	0.416	0.425	0.416	0.405	0.147	Cropping	0.039
	Dry shoots (kg)	0.120	0.099	0.085	0.089	0.085	0.089	0.080	0.046	Cropping	0.062
Bean metrics	Fresh pod mass (g)	0.695	0.510	0.605	0.381	0.605	0.381	0.600	0.170	Cropping	0.038
	Dry pod mass (g)	0.380	0.310	0.180	0.137	0.180	0.137	0.170	0.068	N. S.	N. S.
	Total pod counts (#)	172	100	161	72	161	72	164	33	Cropping	0.041
	Total bean yield mass (g)	3.500	0.368	3.695	0.424	3.695	0.424	3.575	0.410	N. S.	N. S.
Soil water content	SWC at 0-5cm (g)	0.097	0.016	0.135	0.023	0.135	0.023	0.147	0.005	Tillage	0.00014
	SWC at 5-15cm (g)	0.139	0.040	0.093	0.021	0.093	0.021	0.104	0.021	Cropping	< 0.05
	SWC at 15-30cm (g)	0.135	0.027	0.111	0.015	0.111	0.015	0.102	0.016	Cropping	0.073
	SWC at 30-50cm (g)	0.147	0.022	0.131	0.012	0.131	0.012	0.103	0.012	Cropping, Tillage	0.046, 0.053
AMF Colonization	AMF total structures (%)	47.75	35	24.5	13	24.5	13	30.25	23	Cropping	0.041
	AMF Hyphae (%)	27	14	17	8	17	8	20.25	10	Cropping	0.026
	AMF Vesicles (%)	4.25	5	4	5	4	5	2	3	N. S.	N. S.
	AMF Arbuscles (%)	7.5	12	2.75	1	2.75	1	4.5	5	N. S.	N. S.
	AMF Ves + Arb (%)	9	15	0.75	2	0.75	2	3.5	7	N. S.	N. S.