Evaluating Ecosystem and Agronomic Services Provided by Companion Cropping in Hemp (*Cannabis sativa* L.)

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We are nothing without the environment that we inhabit, and this research project would not have been possible without the land that I work with and rely on every day. In our ever-changing climate, research that pushes the norm is not only important, but necessary.

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Abstract

Hemp, *Cannabis sativa* L., was legalized in the 2018 Farm Bill for industrial production. Growing boomed, but profitable agronomic practices still lag in the infant industry. Growers are specifically concerned about weed and pest management strategies that do not impact yield or quality—as there are no certified herbicides or pesticides for hemp— and they want agronomic practices to be sustainable or even regenerative, bettering the land than when they started farming. Our research assesses the utility of companion cropping to address these questions. Companion cropping is a sustainable and cultural management tactic within farming systems in which a secondary crop is planted (in some spatial or temporal configuration) with the main crop, offering an array of potential benefits and ecosystem services. Some of the benefits of companion crops include weed control, increased pollination and habitats for beneficial insects, pest deterrence, increased crop productivity, and soil resilience. To determine which companion crops are most suitable within high cannabinoid hemp production, we have analyzed the effects of intercropping five companions (basil, dill, cilantro, sage, and marigold) on: i) companion plant yield, ii) weed competition, iii) insect diversity, iv) soil microbiome diversity, v) hemp biomass yield, and vi) cannabinoid content. Results show that companion crops differentially and significantly impact weed cover and insect diversity, but do not significantly impact yield or cannabinoid content. This means that growers can choose companion crops that fit their farm and equipment best without having to worry about a negative impact to quality and yield. Future studies will focus on implementation of companion cropping through on farm trials, an exciting and necessary next step to a sustainable future for cannabis production.

Chapter 1:

LITERATURE REVIEW OF CANNABIS, COMPANION CROPPING, AND ECOSYSTEM SERVICES

Cannabis History

Cannabis sativa L. is a dioecious (XY), diploid (2n=2x=10), photoperiod sensitive crop that has become prevalent once again due to recent legalization in the 2018 United States Farm Bill. Cannabis is classified, under current legislation, as either hemp or marijuana based on the amount of tetrahydrocannabinol (THC) that is present: hemp contains less than 0.3% THC; whereas, marijuana contains more than 0.3% THC (Chen & Pan, 2021). Though this is a new crop for modern U.S. growers, cannabis dates back over 6000 years to China, where it was used for textile production, food, and medicine (Li, 1973). These uses reflect the three distinct modern day cannabis products: fiber, grain, and cannabinoids. Cannabis has been grown in other cultures throughout history as well, including Middle Age Scandinavia where it was most likely used to construct ropes and sailcloth (Skoglund et al., 2013). With renewed interest and recent legalization, best practices and potential markets for all types of cannabis are currently being researched (Adesina et al., 2020).

Cannabis Uses

Many parts of the cannabis plant can be utilized including the stalks, seed, and female flowers. The stalks are an excellent source of bast fiber which can be used for ropes, tarps, denim, and textile production (Zimniewska, 2022). The seeds are considered a complete protein, meaning they contain all nine essential amino acids, and can be used for both human and animal consumption, as well as oil production (Zimniewska, 2022). Female flowers are medicinally and recreationally used for cannabinoid production. Cannabidiol (CBD) cannabis has been associated with pain reduction (Miranda-Cortés et al., 2023), epilepsy reduction (Cilio et al., 2014), and Alzheimer's treatment (Campbell & Gowran, 2007). The cannabinoid THC has the potential to treat irritability, anxiety, and stress (Stith et al., 2020). Both phytocannabinoids CBD and THC interact with cannabinoid receptors in the human body (CBR1 and 2). These receptors are part of the endocannabinoid system, and they exist throughout the body (Zou & Kumar, 2018). It is likely through the interaction with these receptors that cannabinoids have had curative or management effects on patients dealing with conditions such as diabetes, Alzheimer's, cancer, and inflammation (Izzo et al., 2009).

United States Cannabis History

Cannabis was grown in the U.S. from the time of colonization until the late 1950s, with most production grown for fiber that was then used for ropes and textiles (Wills, 2021). During this period cannabis was also used medicinally in the U.S., mostly prescribed as a sedative or hypnotic (Kalant, 2001). Despite adoption of cannabis in the U.S., the Marihuana Tax Act was passed in 1937 which placed a tax on the sale of cannabis, and was linked to the fear of "Marijuana" abuse associated with Mexican immigrants (Musto, 1972). This Act caused hemp production to rapidly decline, other than a brief resurgence during World War 2 to assist with rope production for the Navy Fleet, and the last hemp farm shuttered in 1957 (Wills, 2021). In 1970, cannabis became classified as a schedule I drug, a drug that has a high potential for abuse and no medical use, and it became illegal to grow for the next 44 years (APIS). It was not until the 2014 Farm Bill that hemp was once again allowed to be grown in a research capacity and states had to pass independent legislation. Wisconsin Act 100 was passed in 2017 and cultivation began in 2018. Shortly after the 2018 Farm Bill legalized industrial hemp production at the federal level.

Hemp Cultivation

Since hemp has only been grown in the United States for the last decade and in Wisconsin for the last five years, there is much to figure out regarding best production practices. With Wisconsin's rich history in fiber production, being the biggest producer of hemp in the U.S. from the 1920s to the late 1940s (Hildebrandt, 2017), there is a large demand for information from current growers regarding cultivation practices. Hemp is typically dioecious, with separate male and female plants, and is highly heterozygous, which could also cause variation across fields and management techniques (Razumova et al., 2016). As previously discussed, there are three main types of hemp: fiber, grain/seed, and high cannabinoid. Each of these require different cultivation strategies. For grain type hemp, planting is similar to that of corn, where seeds are drilled in rows (Harper et al., 2018). Harvest happens when the seeds begin to shatter and the water content is around 20%; grain combines are typically used for harvesting grain type hemp (Wortmann&Dweikatt, 2020). As for fiber hemp, the seeding rate is generally more than grain hemp due to a need for very tall thin plants, at 40-60 pounds per acre (Conely et al., 2018). Fiber hemp is harvested using a swath or a windrow cut, and it then needs to go through a retting process to separate the bast and hurd fibers (Wortmann&Dweikatt, 2020). Lastly, high-cannabinoid hemp is typically hand planted in rows with 4 foot spacing. They are harvested when the trichomes turn a milky white color, and are typically harvested by hand (Yost et al., 2022).

Hemp Financial Outlook

After the 2018 Farm Bill, hemp production was mostly focused on high cannabinoid hemp, with hemp acreage peaking at 112,204 in 2019. The market demand was not meeting production between 2019 and 2020 which lead to a 62% production drop, as well as a 79% price drop in 2020 (Cruz, 2021). This drop in production can also be linked to stringent compliancy requirements with any plant testing higher than 0.3% THC needing to be destroyed. Alongside cannabinoid floral production, there was 4.37 million pounds of grain hemp valued at 5.99 million dollars, and 33.2 million pounds of fiber hemp valued at 41.4 million dollars grown in 2020 (USDA, 2022). The main restriction that growers face when planting fiber and grain hemp is a lack of processing facilities, so once harvest occurs there are limited places for the hemp to be sent (Cruz, 2021). Even with a lack of processing facilities, many growers are looking to add diversity to their farming operations, and fiber hemp is of great interest (Howard, 2022).

Cropping Systems and Ecosystem Services

Current cultivation practices such as monocropping can lead to poor water quality, soil erosion (Van Duivenbooden et al., 2000), increased pest pressure (Brooker et al., 2015), and elevated CO_2 levels (Bogužas et al., 2022), all leading to decreased yields and increased climate pressure (Smith & Gregory, 2013). There are many alternative cropping systems to this conventional monocropping system that can benefit ecosystem functions, offering ecosystem services such as provisioning, regulating, supporting and cultural services (Gaba et al., 2015) (Figure 1). Maintaining ecosystem services is of utmost importance in regard to sustainability, as according to the millennium ecosystem assessment, many ecosystem services have been irreversibly degraded due to humans and the increased demand for ecosystem goods. With this, large land use changes are necessary to adapt and preserve these necessary ecosystem services. Ecosystem services offer much more than just food: they are integral for climate, flood and disease regulation; fresh water, food and fuel; and they also play a large cultural with respect to spirituality, education, and recreation (Haines-Young & Potschin, 2010). Building this resiliency into cropping systems is essential for continued food production, especially given the ever-increasing global population and a need for global food security with extreme weather events (Mehrabi et al., 2022). Companion cropping can offer many ecosystem services such as increased pollinators, yield gains (Griffiths-Lee et al., 2020), long-term soil fertility, clean water through filtration, and biological control among others (Philip Robertson et al., 2014).

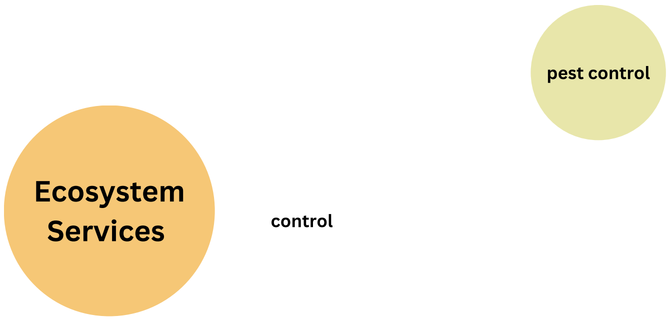


Figure 1*:* Ecosystem services: periwinkle is provisioning; light yellow is regulation; pink is supporting; green is cultural. Adapted from: https://www.nature.scot/scotlands-biodiversity/scottish-biodiversity-strategy-and-cop15/ecosystem-approach/ecosystem-services-natures-benefits.

Soil Health and Ecosystem Services

Ecosystem services heavily rely on soil health, and loss of these services greatly affects growers all over the world (Swift, 2006). Actively working on restoring soil health through practices such as conservation agriculture, with intercropping being one of these practices, has been shown to contribute to increasing ecosystem services (Blum, 2005). Intercropping is a sustainable agriculture practice where two or more species are grown together to strengthen the agroecosystem (Hiss et.al., 2022). By applying these conservation techniques, there is a 28-500% decrease in soil erosion, and 28-50% less runoff of harmful chemicals (Kihara et al., 2020). Intensive farming has led to decreased nutrient use efficiency and an increase of environmental pressures due to high inputs such as chemical fertilizers and pesticides (Zhang et al., 2010). This decreased nutrient use efficiency has a direct correlation to decreased rhizosphere processes such as enzyme secretion and mobilization of nutrients (Drinkwater & Snapp, 2007). Microbes, in the rhizosphere and outside of it, play a large role in soil function as well. Microorganisms are responsible for many cycles that promote plant growth such as mineralization, fixation, and mobilization of nutrients, as well as many forming symbiotic relationships with plants to further increase nutrient supplies (Suman et al., 2022). Intensive farming can lead to the destruction of these valuable organisms, as it destroys soil organic matter, soil structure, and increases soil erosion. Though these growing practices have led to an increase in food production, they are ultimately depleting the very soil they need, and the ecosystem services that come along with it (Kughur, 2015).

Companion cropping history

Most of the knowledge that is available on alternative cropping systems is not in peer-reviewed literature but found in traditional ecological indigenous knowledge. Companion cropping is an important cropping system to indigenous communities as demonstrated by the practice of the three sisters—maize, beans, and squash. The main idea is that the beans can use maize as a climbing pole while offering nitrogen through rhizobia bacteria, as the large squash leaves spread across the ground and smother weeds (Woolley & Davis, 1991). It was believed that these plant characteristics made growing these three crops together beneficial for long term soil health and yield. This form of companion cropping also allowed for settlement in what were typically nomadic communities, and formed sedentary crop development, as well as dependency on this crop cultivation method (Hart & Rieth, 2002). Indigenous agriculture views all parts of the land as necessary, whether that be weeds or pests, and to use chemicals to get rid of some element of the environment is antagonistic to nature. Since humans are a part of the environmental system, it is up to farmers to adjust to the environment, not force the environment to adjust to them (Mangan, n.d). Companion cropping is more than just planting crops together, it is the idea that these crops can work with the environment to allow for both agriculture and ecology to coexist and benefit from each other, something that is not found in many modern production systems.

Common companion crops

Another key aspect of companion cropping is choosing crops that work well with the main crop. Plant phenology, size, secondary metabolites, field layout, harvest timeline, pest pressure, and nutrient needs are all important considerations. Increasing diversity through companion cropping allows for possible pest protection, weed suppression, increased soil health, increased shade cover, and overall better use of space (Harris & Streets, 2022). By using more space, productivity increases, as intercropped systems, on average, produce 1.7 times more biomass than monocropped systems (Cardinale et al., 2007). As far as pests go, it is not wise to plant a crop from the same family as the main crop, as families typically share pests. A common benefit of companion cropping is pest reduction, so planting crop relatives would do the exact opposite. For example, cannabis and hops (*Humulus* genus) are closely related (McPartland, 2018). This close relation means that these crops share pests, so it would not make sense to plant those two next to each other as it could lead to increased pest pressure for both crops. An example of why this is important would be in the case of powdery mildew. *P. macularis* is a pathogen that infects hops. This genus of powdery mildew has now been reported in hemp fields across the Pacific North West (Punja, 2022).

Nutrient needs are also essential to consider. Planting two crops that both need a large amount of nitrogen input may not make much sense, because companion cropping should lead to decreased inputs (i.e. one plant requires less of one nutrient than the other, so one is able to take up that nutrient more easily with less outside inputs necessary). This is demonstrated in the three sisters, where one plant—typically a legume— fixes nitrogen for the others to uptake (Altieri et al., 2012).

There are many traits that growers may look for when choosing a companion crop based off of specific field concerns. If soil health is of concern, it may be best to plant crops with taproots or deep roots in order to break up compaction and find hard to access nutrients (Dalman, 2022). Herbs are well known companion crops for their fragrant scent and essential oil content, which ends up deterring many pests, or attracting pollinators (Walliser, 2021). For our project, five companion crops were chosen—dill, cilantro, basil, sage, and marigold. Dill and sage are well known for their scent that deters pests. Dill, cilantro, and basil are all flowering herbs, which increases pollination, along with dill and cilantro boasting umbrella flower structures, which offer hiding spots for predatory insects. Marigolds are one of the most common companion plants as their scent upsets pests, their flowers increase pollination, and they are fast and large growers (Walliser, 2021). There were a plethora of other companion plants that could have been chosen, but what set our selected plants apart was their growth period and growth habit. Dill and cilantro are fast growing herbs, which develop quickly due to a need for plentiful sun, and can be harvested before surrounding hemp plants become too large and shade them out (Trinklein, 2022). Sage is a perennial, and is able to do well with limited direct light—though prefers sun—, meaning it is able to still grow while the hemp is large and close to harvest, and will continue to grow well afterwards. It was also selected because it is a sacred plant to our collaborators at Lac Courte Oreilles Ojibwa Community College. Basil and marigold both establish and grow large very quickly, which makes them perfect candidates for weed control (University of Minnesota Extension). On top of this, basil produces very quickly, and can be harvested multiple times throughout its growing cycle having high protentional as a secondary income source (Pearson, 2020).

Research Partnership

This research study was in partnership with the Lac Courte Oreilles Ojibwe College (LCOOC), and the funding comes from the USDA NIFA Tribal Colleges Research Grant Program. This companion cropping study was designed in collaboration with LCOOC as they wanted to bring hemp cultivation to their reservation in a sustainable fashion. They have a steadfast commitment to the integrity of their land, so an alternative cropping system that supports ecosystem services was mandatory when developing this project. This companion study was implemented by following key mission to find an alternative cropping system for hemp that could introduce possible best practices that work with the land.

Agroecology as a social and environmental movement

Our study is rooted in agroecology. First, we are looking for best production practices that do not negatively impact yield. The current U.S. agriculture system is heavily focused on yield and high output systems—which typically correlate to high input systems. In order to make companion cropping attractive to farmers, yields need to stay relatively similar, because few people will be able to sacrifice money in an already limited income profession. This puts growers in a hard spot, as they care about their land but also have to make a profit. This is where companion cropping can become more appealing. Companion cropping offers income in multiple ways—by the main crop, being cannabis in this case, and the secondary crop(s). Depending on what equipment growers already have, and their scale of production, companion cropping can benefit the farmer through increased yield, lower inputs, and crop diversification. As for the environment, companion cropping can reduce pest, weed, and disease pressure, improve soil fertility and offer erosion control, and increase pollination (Walliser, 2021). All of these factors relate to ecosystem services, which were previously discussed, which directly relate to the health of the environment.

Agroecology is the perfect discipline for establishing these novel cropping systems due to its transdisciplinary approach to agriculture. There are three tenets to agroecology: agronomy, environmental science, and social science. Each of these three disciplines needs to have equal pull in whatever research is being done, as agroecology takes a systems approach, focusing on agricultural production systems and how they interact with the environment and people (Brym & Reeve, 2016). Agroecology is not only focused on growing food, but on reducing environmental pressures on earth: decreasing renewable resources, ongoing malnutrition, poverty, climate change, and a loss of biodiversity (IPBES, 2019; IPCC, 2021). The Special Rapporteur, individuals appointed by the United Nations to report on human rights issues around the world, has identified agroecology as the approach to agriculture that can increase yields, bring positive progress to groups experiencing malnutrition, and aid the environment (Schutter, 2010). The UN has organized many meetings and symposiums to discuss the vitality of agroecology and how to scale it up to meet current food demands.

Agroecology is a social movement. It is a way for people to organize and take charge of their community, food sovereignty, and environment. Agroecology aims at building culturally relevant food systems that bring economic independence, while supporting local farmers, rural communities, and local/ indigenous knowledge (Altieri & Toledo, 2011). This political and social aspect of agroecology is essential to the movement, as it is hopes to aid in problems that directly relate to humans, making it innately political. It is this holistic approach that sets agroecology apart from others. It is a transdisciplinary approach to food system change that involves everyone affected, and actively pushes against political, economic, and social power structures (Gliessman, 2018; Wezel et al., 2020).

In the context of companion cropping, agroecology offers the ability to bring economic sovereignty to growers by increasing outputs. Agroecological practices can also positively impact the environment by increasing ecosystem services, increasing soil microbial diversity, and decreasing pest pressure. This study in particular takes in the social approach as well, with the Lac Courte Oreilles Ojibwa Tribe wanting economic independence through growing, processing, and selling hemp, pushing against the current power structures that are in place. Agroecology's holistic approach is fundamental to this project, as one tenet is not more important than another; they are all interlocking pieces that are necessary to power the change, both socially and environmentally, that is needed in order to change the current food system and establish best practices that are positive for growers and the land they use.

Research Focus

Our research focused on assessing the utility of companion cropping in cannabis. Our goal was to research best production practices that do not sacrifice yield and offer a possible secondary source of income for growers. To do this we assessed five companion crops for their ability to suppress weeds, and monitored hemp and companions for both beneficial and harmful insects. We hypothesized that the addition of companion crops to a hemp production system would increase diversity of beneficial insects, suppress weeds, deter pests from the main crop, and offer financial incentive for growers looking to diversify their field. Our preliminary results found that fast growing, high biomass companion crops such as marigold and basil performed better as they took up more space between rows, therefore outcompeting weeds and increasing surface area for potential beneficial insects to land. They also did not impact the yield of the main crop, and did not negatively impact the soil microbiome.

References

Adesina, I., Bhowmik, A., Sharma, H., & Shahbazi, A. (2020). A Review on the Current State of Knowledge of Growing Conditions, Agronomic Soil Health Practices and Utilities of Hemp in the United States. *Agriculture*, *10*(4), 129. https://doi.org/10.3390/agriculture10040129

Altieri, M. A., Funes-Monzote, F. R., & Petersen, P. (2012). Agroecologically efficient agricultural systems for smallholder farmers: contributions to food sovereignty. *Agronomy for Sustainable Development*, *32*(1), 1–13. https://doi.org/10.1007/s13593-011-0065-6

Altieri, M. A., & Toledo, V. M. (2011). The agroecological revolution in Latin America: rescuing nature, ensuring food sovereignty and empowering peasants. *Journal of Peasant Studies*, *38*(3), 587–612. https://doi.org/10.1080/03066150.2011.582947

Blum, W. E. (2005). Functions of soil for society and the environment. *Reviews in Environmental Science and Bio/Technology*, *4*, 75-79.

Bogužas, V., Skinulienė, L., Butkevičienė, L. M., Steponavičienė, V., Petrauskas, E., & Maršalkienė, N. (2022). The Effect of Monoculture, Crop Rotation Combinations, and Continuous Bare Fallow on Soil CO2 Emissions, Earthworms, and Productivity of Winter Rye after a 50-Year Period. *Plants*, *11*(3), 431. https://doi.org/10.3390/plants11030431

Bridgeman, M. B., & Abazia, D. T. (2017). *Medicinal Cannabis: History, Pharmacology, And Implications for the Acute Care Setting*. *42*(3).

Brooker, R. W., Bennett, A. E., Cong, W., Daniell, T. J., George, T. S., Hallett, P. D., Hawes, C., Iannetta, P. P. M., Jones, H. G., Karley, A. J., Li, L., McKenzie, B. M., Pakeman, R. J., Paterson, E., Schöb, C., Shen, J., Squire, G., Watson, C. A., Zhang, C., … White, P. J. (2015). Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, *206*(1), 107–117. https://doi.org/10.1111/nph.13132

Brym, Z. T., & Reeve, J. R. (2016). *Agroecological Principles from a Bibliographic Analysis of the Term Agroecology* (pp. 203–231). https://doi.org/10.1007/978-3-319-26777-7_5

Campbell, V. A., & Gowran, A. (2007). Alzheimer's disease; taking the edge off with cannabinoids? *British Journal of Pharmacology*, *152*(5), 655–662. https://doi.org/10.1038/sj.bjp.0707446

Cardinale, B. J., Wright, J. P., Cadotte, M. W., Carroll, I. T., Hector, A., Srivastava, D. S., Loreau, M., & Weis, J. J. (2007). Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proceedings of the National Academy of Sciences*, *104*(46), 18123–18128. https://doi.org/10.1073/pnas.0709069104

Chen, C., & Pan, Z. (2021). Cannabidiol and terpenes from hemp – ingredients for future foods and processing technologies. *Journal of Future Foods*, *1*(2), 113–127. https://doi.org/10.1016/j.jfutfo.2022.01.001

Cilio, M. R., Thiele, E. A., & Devinsky, O. (2014). The case for assessing cannabidiol in epilepsy. *Epilepsia*, *55*(6), 787–790. https://doi.org/10.1111/epi.12635

Cruz, J. C., House, L. A., & Blare, T. D. (2021). Global overview of hemp production and the market of hemp-derived CBD in the US. 2021 Annual Meeting, August 1-3, Austin, Texas 31297, Agricultural and Applied Economics Association. DOI: 10.22004/ag.econ.312927

Dalman, N. (2022). *Marigolds*. UMN Extension. https://extension.umn.edu/flowers/marigolds

Drinkwater, L. E., & Snapp, S. S. (2007). *Nutrients in Agroecosystems: Rethinking the Management Paradigm* (pp. 163–186). https://doi.org/10.1016/S0065-2113(04)92003-2

Conely, S., Gaska, J., Roth, A., Skjolaas, C., Silva, rin, Ortiz-Ribbing, L., Barker, W., & Robinson, P. (2018). *Industrial hemp agronomics*. Wisconsin Hemp. https://fyi.extension.wisc.edu/hemp/industrial-hemp-agronomics/

Gaba, S., Lescourret, F., Boudsocq, S., Enjalbert, J., Hinsinger, P., Journet, E.-P., Navas, M.-L., Wery, J., Louarn, G., Malézieux, E., Pelzer, E., Prudent, M., & Ozier-Lafontaine, H. (2015). Multiple cropping systems as drivers for providing multiple ecosystem services: from concepts to design. *Agronomy for Sustainable Development*, *35*(2), 607–623. https://doi.org/10.1007/s13593-014-0272-z

Gliessman, S. (2018). Defining Agroecology. *Agroecology and Sustainable Food Systems*, *42*(6), 599–600. https://doi.org/10.1080/21683565.2018.1432329

Griffiths-Lee, J., Nicholls, E., & Goulson, D. (2020). Companion planting to attract pollinators increases the yield and quality of strawberry fruit in gardens and allotments. *Ecological Entomology*, *45*(5), 1025–1034. https://doi.org/10.1111/een.12880

Haines-Young, R., & Potschin, M. (2010). The links between biodiversity, ecosystem services and human well-being. In *Ecosystem Ecology* (pp. 110–139). Cambridge University Press. https://doi.org/10.1017/CBO9780511750458.007

Harper, J., Alyssa, C., Kime, L., Roth, G., & Manzo, H. (2018). *Industrial hemp production*. Penn State Extension. https://extension.psu.edu/industrial-hemp-production

Harris, N., & Streets, J. (2022). *Companion planting*. Extension. https://extension.wvu.edu/lawn-gardening-pests/gardening/garden-management/companion-planting#:~:text=Companion%20planting%20allows%20you%20to,deeper%20in%20the%20soil%20profile.

Hart, J. P., & Rieth, C. B. (2002). Maize and Villages: A Summary and Critical Assessment of Current Northeast Early Late Prehistoric Evidence. In *Northeast subsistence-settlement change, A.D. 700-A.D. 1300* (pp. 342–358). essay, New York State Museum/New York State Education Department.

Hildebrandt, D. (2017). *Hemp: Wisconsin's forgotten harvest*. Wisconsin Historical Society. https://content.wisconsinhistory.org/digital/collection/wmh/id/52526.

Howard, J. (2022). *Fiber hemp: Haute or hype?*. Fiber Hemp: Haute or Hype? | Crop and Soil Sciences | NC State University. https://cals.ncsu.edu/crop-and-soil-sciences/news/fiber-hemp-haute-or-hype/

Huss, C; Holmes, K; Bludaugh, C. (2022). Benefits and Risks of Intercropping for Crop Resilience and Pest Management. *Journal of Economic Entomology*. doi.org/10.1093/jee/toac045

IPBES (2019). Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. E. S. Brondizio, J. Settele, S. Díaz, and H. T. Ngo (editors). IPBES secretariat, Bonn, Germany. 1148 pages. https://doi.org/10.5281/zenodo.3831673

IPCC (2022). Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group 11 to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press. Cambridge University Press, Cambridge, UK and New York, NY, USA, 3056 pp., doi:10.1017/9781009325844.

Izzo, A. A., Borrelli, F., Capasso, R., Di Marzo, V., & Mechoulam, R. (2009). Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends in Pharmacological Sciences*, *30*(10), 515–527. https://doi.org/10.1016/j.tips.2009.07.006

Kalant, H. (2001). Medicinal Use of Cannabis: History and Current Status. *Pain Research and Management*, *6*(2), 80–91. https://doi.org/10.1155/2001/469629

Kihara, J., Bolo, P., Kinyua, M., Nyawira, S. S., & Sommer, R. (2020). Soil health and ecosystem services: Lessons from sub-Sahara Africa (SSA). *Geoderma*, *370*, 114342. https://doi.org/10.1016/j.geoderma.2020.114342

Li, H.-L. (1973). An archaeological and historical account of cannabis in China. *Economic Botany*, *28*(4), 437–448. https://doi.org/10.1007/BF02862859

Mangan, A. (n.d). Decolonizing Regenerative Agriculture: An Indigenous Perspective. *Bioneers*.

McPartland, J. M. (2018). *Cannabis* Systematics at the Levels of Family, Genus, and Species. *Cannabis and Cannabinoid Research*, *3*(1), 203–212. https://doi.org/10.1089/can.2018.0039

Mehrabi, Z., Delzeit, R., Ignaciuk, A., Levers, C., Braich, G., Bajaj, K., Amo-Aidoo, A., Anderson, W., Balgah, R. A., Benton, T. G., Chari, M. M., Ellis, E. C., Gahi, N. Z., Gaupp, F., Garibaldi, L. A., Gerber, J. S., Godde, C. M., Grass, I., Heimann, T., … You, L. (2022). Research priorities for global food security under extreme events. *One Earth*, *5*(7), 756–766. https://doi.org/10.1016/j.oneear.2022.06.008

Miranda-Cortés, A., Mota-Rojas, D., Crosignani-Outeda, N., Casas-Alvarado, A., Martínez-Burnes, J., Olmos-Hernández, A., Mora-Medina, P., Verduzco-Mendoza, A., & Hernández-Ávalos, I. (2023). The role of cannabinoids in pain modulation in companion animals. *Frontiers in Veterinary Science*, *9*. https://doi.org/10.3389/fvets.2022.1050884

Musto, D. F. (1972). The Marihuana Tax Act of 1937. *Archives of General Psychiatry*, *26*(2), 101. https://doi.org/10.1001/archpsyc.1972.01750200005002

Pearson, L., & Drost, D. (2020). *Basil in the garden*. USU. https://extension.usu.edu/yardandgarden/research/basil-in-the-garden

Philip Robertson, G., Gross, K. L., Hamilton, S. K., Landis, D. A., Schmidt, T. M., Snapp, S. S., & Swinton, S. M. (2014). Farming for Ecosystem Services: An Ecological Approach to Production Agriculture. *BioScience*, *64*(5), 404–415. https://doi.org/10.1093/biosci/biu037

Pickett, J. A., Midega, C. A., Pittchar, J., & Khan, Z. R. (2019). Removing constraints to sustainable food production: new ways to exploit secondary metabolism from companion planting and GM. *Pest Management Science*, ps.5508. https://doi.org/10.1002/ps.5508

Punja, Z. K. (2022). First report of the powdery mildew pathogen of hops, *Podosphaeria macularis* , naturally infecting cannabis (*Cannabis sativa* L., marijuana) plants under field conditions. *Canadian Journal of Plant Pathology*, *44*(2), 235–249. https://doi.org/10.1080/07060661.2021.1960424

Razumova, O. V., Alexandrov, O. S., Divashuk, M. G., Sukhorada, T. I., & Karlov, G. I. (2016). Molecular cytogenetic analysis of monoecious hemp (Cannabis sativa L.) cultivars reveals its karyotype variations and sex chromosomes constitution. *Protoplasma*, *253*(3), 895–901. https://doi.org/10.1007/s00709-015-0851-0

Ren, M., Tang, Z., Wu, X., Spengler, R., Jiang, H., Yang, Y., & Boivin, N. (2019). The origins of cannabis smoking: Chemical residue evidence from the first millennium BCE in the Pamirs. *Science Advances*, *5*(6), 1391–1403. https://doi.org/10.1126/SCIADV.AAW1391/SUPPL_FILE/AAW1391_SM.PDF

Schutter, O. D. (2010). (rep.). *Human Rights Council*. United Nations.

Skoglund, G., Nockert, M., & Holst, B. (2013). Viking and Early Middle Ages Northern Scandinavian Textiles Proven to be made with Hemp. *Scientific Reports*, *3*(1), 2686. https://doi.org/10.1038/srep02686

Smith, P., & Gregory, P. J. (2013). Climate change and sustainable food production. *Proceedings of the Nutrition Society*, *72*(1), 21–28. https://doi.org/10.1017/S0029665112002832

Stith, S. S., Li, X., Diviant, J. P., Brockelman, F. C., Keeling, K. S., Hall, B., & Vigil, J. M. (2020). The effectiveness of inhaled Cannabis flower for the treatment of agitation/irritability, anxiety, and common stress. *Journal of Cannabis Research*, *2*(1), 47. https://doi.org/10.1186/s42238-020-00051-z

Suman, J., Rakshit, A., Ogireddy, S. D., Singh, S., Gupta, C., & Chandrakala, J. (2022). Microbiome as a Key Player in Sustainable Agriculture and Human Health. *Frontiers in Soil Science*, *2*. https://doi.org/10.3389/fsoil.2022.821589

Swift, M. J., Stroud, K., Shepherd, A., Albrecht, A., Bationo, P., Mafongoya, F., ... & Walsh, M. (2006). Confronting land degradation in Africa: challenges for the next decade. *World agroforestry into the future. World Agroforestry Centre, Nairobi, Kenya*, 43-51.

Teoh, E. S. (2016). Secondary Metabolites of Plants. In *Medicinal Orchids of Asia* (pp. 59–73). Springer International Publishing. https://doi.org/10.1007/978-3-319-24274-3_5

USDA. (2022). (rep.). *National Hemp Report*. USDA.

Trinklein, D. (2022). *Growing herbs at home*. University of Missouri Extension. https://extension.missouri.edu/g6470

Van Duivenbooden, N., Pala, M., Studer, C., Bielders, C. L., & Beukes, D. J. (2000). Cropping systems and crop complementarity in dryland agriculture to increase soil water use efficiency: a review. *NJAS: Wageningen Journal of Life Sciences*, *48*(3), 213–236. https://doi.org/10.1016/S1573-5214(00)80015-9

Walliser, Jessica. *Plant Partners: Science-Based Companion Planting Strategies for the Vegetable Garden: Science-Based Companion Planting Strategies for the Vegetable Garden*. Storey Publishing, 2021.

Wezel, A., Herren, B. G., Kerr, R. B., Barrios, E., Gonçalves, A. L. R., & Sinclair, F. (2020). Agroecological principles and elements and their implications for transitioning to sustainable food systems. A review. *Agronomy for Sustainable Development*, *40*(6), 40. https://doi.org/10.1007/s13593-020-00646-z

WillsT, M. (2021). *The return of hemp - JSTOR DAILY*. The Return of Hemp. https://daily.jstor.org/the-return-of-hemp/

Woolley, J., & Davis, J. H. C. (1991). The agronomy of intercropping with beans. *Common beans: research for crop improvement.*, 707-735.

Wortmann, C., & Dweikat, I. (2020, March 26). *Hemp production for fiber or grain - revised*. Hemp Production for Fiber and Grain. https://cropwatch.unl.edu/2019/hemp-production-fiber-or-grain

Yost, M., Baker, M., Sullivan, T., Gale, J., Zesiger, C., Creech, E., & Bugbee, B. (2022). *Ten frequently asked questions about growing cannabis*. Ten Frequently Asked Questions About Growing Cannabis. https://extension.usu.edu/crops/research/ten-frequently-asked-questions-about-growing-cannabis

Zhang, F., Shen, J., Zhang, J., Zuo, Y., Li, L., & Chen, X. (2010). *Rhizosphere Processes and Management for Improving Nutrient Use Efficiency and Crop Productivity* (pp. 1–32). https://doi.org/10.1016/S0065-2113(10)07001-X

Zimniewska, M. (2022). Hemp Fibre Properties and Processing Target Textile: A Review. *Materials*, *15*(5), 1901. https://doi.org/10.3390/ma15051901

Zou, S., & Kumar, U. (2018). Cannabinoid Receptors and the Endocannabinoid System: Signaling and Function in the Central Nervous System. *International Journal of Molecular Sciences*, *19*(3), 833. https://doi.org/10.3390/ijms19030833

Chapter 2: COMPANION CROPPING IN HEMP *(Cannabis sativa L.)* FOR INCREASED ECOSYSTEM SERVICES AND SUSTAINBALE BEST PRACTICES

Abstract

With the relegalization of hemp (*Cannabis sativa* L.) in 2018, came a stark increase in production. This increase led to growers across the U.S. lacking best management practices in the infant industry. Top concerns for growers are weed and pest management, yield, and sustainable practices. With no certified synthetic herbicides or pesticides, growers are left to find other ways to manage these issues, typically resulting in increased labor and cost. Along with this, the hemp market is currently experiencing a large amount of biomass supply mixed with a demand that does not match. To combat these issues, our research study has implemented companion cropping in hemp. Companion cropping is a cultural practice that involves planting multiple crops together in order to offer ecosystem services, and increase productivity, yield, and crop, insect, and soil microbiome diversity Some ecosystem services and benefits of companion cropping include weed control, increased pollination and habitats for beneficial insects, pest deterrence, increased crop productivity, soil resilience in the face of climate change, and erosion control. We have planted and analyzed five common companion crops—dill, cilantro, sage, marigold, and basil— on: i) companion plant yield, ii) weed competition, iii) insect diversity, iv) soil microbiome diversity, v) hemp biomass yield, and vi) cannabinoid content. Our results suggest that companion crops differentially and significantly impact weed cover, insect species, and companion price, but do not impact plant height, yield, or cannabinoid content. Future studies should focus on expansion of this project through on farm trials, as well as long term soil assessments, in order to better understand how companion cropping in cannabis affects ecosystem services long term.

Introduction

Hemp (*Cannabis sativa* L). has regained popularity in the United States due to its recent relegalization in the 2018 Farm Bill. With this interest came a boom of high cannabinoid hemp production, peaking in 2019 with 112,204 acres planted. Strict regulations and increased supply coupled with decreased demand led to the cost of hemp drastically dropping: with a 79% price drop coupled with a 62% production drop (Cruz, 2021). Another key difficulty growers face is a lack of federally certified chemical options, so they need to find alternative ways to deal with pest and weed pressure. Even with the reduction in production and price, there was still 19.7 million pounds of high cannabinoid hemp grown in 2021 (USDA, 2022). This indicates that growers are still interested in producing cannabis, but may need additional financial incentives and good production practices to keep growing.

Current high cannabinoid hemp production practices echo typical United States monocropping—rows of hemp with bare alleyways. Though monocropping is the typical production practice for crops in the U.S., it can lead to poor water quality, soil erosion, increased pest pressure, elevated CO_2 levels, decreased yields, and increased climate pressure (Van Duivenbooden et al., 2000; Brooker et al., 2015; Bogužas et al., 2022; Smith & Gregory, 2013). A shift in production practices is necessary to ensure longevity and health of the land. This shift to more sustainable production practices will also include protection of ecosystem services including nutrient cycling, erosion control, pollinator habitat, carbon storage, and pest control among many others (Griffiths-Lee et al., 2020; Philip Robertson et al., 2014) (Figure 1). Diversifying the typical monocropping production practice will also increase resiliency, which is specifically important to safeguard food security in face of climate change and increased extreme weather events (Mehrabi et al., 2022).

Figure 1: With ecosystem services on the y-axis and ecological health on the x-axis, companion cropping is high on both, with increased ecosystem services and ecological health; whereas, monoculture farming falls low on this, having both decreased ecosystem services and ecological health. Figure adapted from: http://devp-service.oss-cn-beijing.aliyuncs.com/8e9430f7d6f94fd5a5d3b233b0355a5f/file_1639100997672.pdf

Companion cropping is one alternative cropping system that could help build resiliency within hemp production. Companion cropping has been practiced since the advent of the three sisters, an indigenous way of planting that includes planting maize, beans, and squash together with the idea that each of these crops offers the others a benefit from weed control, stability, and nitrogen fixation (Woolley & Davis, 1991). The three sisters show the important interactions that plants can have with each other, and that thoughtfully planned cropping systems do not need external inputs.

Companion cropping is anti-artificial input in nature, as indigenous agriculture views all parts of the environment as necessary and strives to find ways to work with the environment to address issues like increased pest pressure. This is done by not relying on spraying and chemical control but instead looking at alternative systems that work with the land. Companion cropping and indigenous agriculture are about adjusting to the environment that is being used, not forcing it to adjust to modern agriculture production (Mangan, n.d.). Recent companion cropping studies show that this increase in plant diversity can act as a barrier for disease spread, can decrease the abundance of invasive pests, and increases plant productivity by increasing beneficial microbes in soil (Marzani, 2023; Peter et al., 2023; Lan et al., 2023). Companion cropping studies go hand in hand with indigenous knowledge with shared goal of creating sustainable cropping systems that are resilient and able to face current climate struggles head on.

By increasing diversity through companion cropping there is possible pest protection, weed suppression, increased soil health, increased shade cover, and an overall better use of space by decreased bare soil (Harris & Streets, 2022). These traits lead to an increase in productivity with intercropped systems producing 1.7 times more biomass that monocropped systems (Cardinale et al., 2007). Studies have also found companion cropping to significantly decrease weed cover and increase biomass and yield (Verret et al., 2017). For cannabis growers, this is especially important given the decrease in price of hemp. Biomass and cannabinoids, in the form of a female inflorescence, are the two main components of yield. An increase in biomass production could mean in increase in profits for many growers, which is essential when establishing best and lasting practices for hemp growers. Companion cropping also offers possible secondary profit to growers in the early season, as companion crops are harvested earlier in the season when labor needs are lower.

This research is in partnership with the Lac Courte Oreilles Ojibwe College, and was brought forward by the college due to their want of bringing hemp cultivation to their reservation.

A study that commits to maintaining the integrity of their land was essential, so finding production practices that support ecosystem services and work with the land was paramount in developing project objectives. At its core, this study is rooted in indigenous knowledge and agroecological practices. It is designed to work alongside growers and find solutions to everyday problems that they face; problems such as pest and weed pressure that would typically be dealt with by applying synthetic chemicals. This study aims to develop a healthy farming system that decreases the need for outside inputs. It is also focused on the transdisciplinary nature of agroecology, being the meeting point of agronomy, ecology, and sociology, by focusing on agricultural production systems and how they interact with the environment and people (Brym & Reeve, 2016). Agroecology has been identified as the approach to agriculture that has the ability to bring about positive progress to issues such as malnutrition, climate change, and food sovereignty (Schutter, 2010).

The aim of our research was to quantify the positive and negatives attributes of companion cropping in cannabis and to make recommendations for best companions to utilize. To do this we intercropped five companion crops and compared them to each other and the standard monocropping method for weed suppression, beneficial and harmful insects and microbial communities, and yield. We hypothesized that the addition of companion crops to a hemp production system would increase diversity of beneficial insects, suppress weeds, deter pests from the main crop, and offer financial incentive for growers looking to diversify their field. More prolific companion crops such as marigold and basil will perform better as they take up more space between rows, therefore outgrowing weeds and increasing surface area for insects. This research will offer potential best practices for a sustainable future for hemp production.

Materials and Methods

Experimental Design and Plant Materials

Field trials were conducted at the UW Madison, West Madison Agricultural Research Station, (43.06424, -89.53444) (2021 and 2022), as well as in Hayward, Wisconsin, (45.98820, -91.37825), in collaboration with the Lac Courte Oreilles Ojibwe University (2021). A randomized complete block design with four blocks and six companion plant treatments (basil, cilantro, dill, marigold, sage, and a no companion control) was utilized in 2021 and 2022 at West Madison. A RCBD with three blocks was used at LCO in 2021. The feminized (female-only) hemp cultivar, Cherry Wine (Fortuna Hemp), was used at all locations/years. All plants, companions and hemp, were directed seeded into 72-cell plastic trays in the UW Madison Walnut Street Greenhouse and transplanted into the field by hand. Seeds were planted in the greenhouse on May 15th and May 20th in 2021 and 2022, respectively. Hemp plants were transplanted June 8th 2021 (West Madison), June 15th 2021 (LCO), and June 16th 2022 (West Madison) into 4' wide black plastic mulch with a 4' within row spacing and ~4' between row spacing. Companion crops were transplanted in the alleys in 20-foot rows according to recommendations on the seed packets: marigolds and sage were planted at one foot spacing, and cilantro, dill, and basil were planted six inches apart (Figure 2). A border of hemp plants was planted around the perimeter of the experiment. In 2021, both sites received a layer of straw mulch between rows to suppress weeds as well as increase soil moisture holding capacity (Picture 1a). This straw was not a part of the 2022 experiment. Neither of the planting locations received season long irrigation, but all three replications were watered for the first two weeks to assure that companions and hemp were able to establish after transplanting (Picture 1b). No fertilization was used during the experiment.

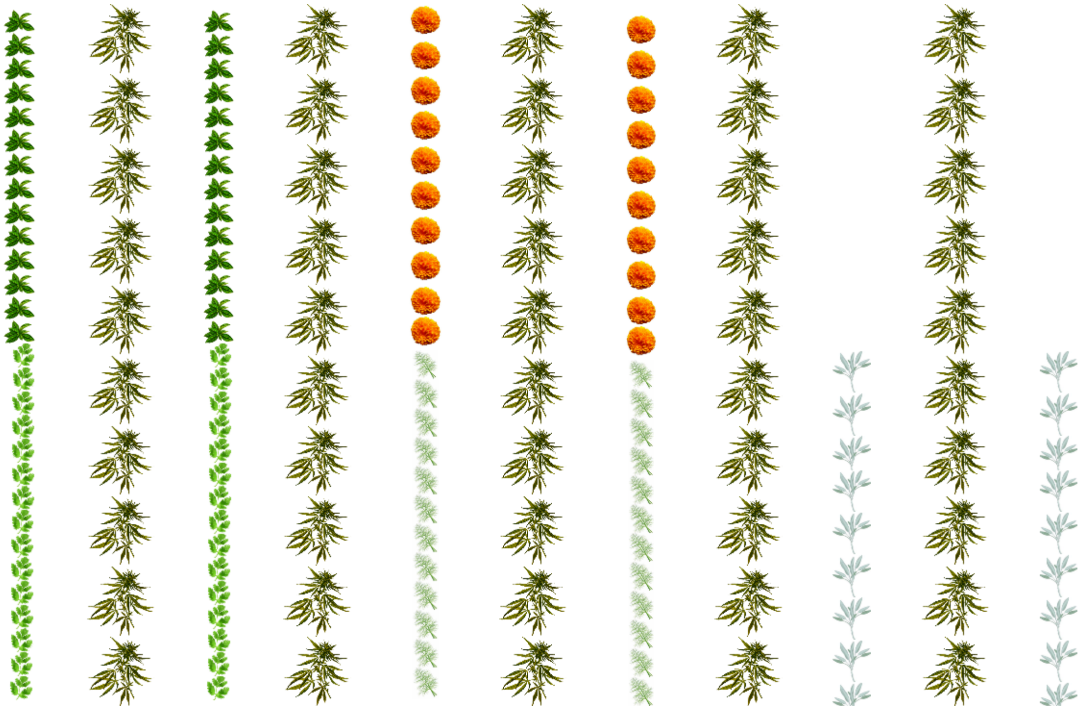


Figure 2: Field layout diagram illustrating 1 block. Each hemp plant represents four feet, with each companion treatment surrounding the hemp in 20’ plots. Basil, cilantro, and dill plants were planted every six inches, while marigold and sage were planted every 12 inches. The blank treatment is shown in the top right.

Picture 1: a) Field conditions at LCO in 2021. b) Field conditions at WMARS in 2021.

Trait Collection

Weed cover was taken 15-20 days post transplanting at each location. Weed cover was assessed by using a one by one-foot quadrat and randomly sampling three locations within each companion treatment. Weed percent was assessed by comparing weed cover, companion cover, and ground cover in each quadrat. These traits were each assigned a percentage of the quadrat, and weed cover was calculated as a percentage. All data was recorded in the field using the Field Book application to avoid human error and help with downstream processing (Rife & Poland, 2014).

Companion Yield and Insect Diversity

Companion crops were harvested when salable—between 3 to 10 weeks after planting depending on crop, with basil being harvested twice. Harvested companions were weighed and then price according to market rate by averaging prices from local farmers markets and using a "bunch" measurement, which is agreed to be equivalent to 2 ounces. Half of a companion treatment (10ft) was left in the field to assess insect diversity after flowering. Insect samples were taken at two timepoints, one month after transplanting, and one week prior to harvest. Twenty sweeps were taken in each treatment, sweeping both the hemp and companion crop for a cumulative sample. Insects were kept in a -20°C freezer, and then characterized at the family level. Each insect was given a classification of a potential beneficial, potential pest, or incidental according to reference manuals Natural Enemies Handbook, Hemp Diseases and Pests, and Biological Control of Insects and Mites, and verified by UW Madison Extension Entomologist Patrick Liesch, MS (Mahr & Ridgway, 1993; Watson et al., 2000; Flint & Dreistadt, 1998).

Microbiome

Soil samples were collected halfway through the growing season at the West Madison site in both 2021 and 2022. Three soil cores were taken from each treatment (15 cm depth), placed in a bucket and mixed together for one homogenized sample per plot. Samples were stored at 4°C, and then processed through a 2mm sieve. 250 mg of soil was weighed into a 2ml tube, and microbial DNA was extracted using the DNeasy power soil pro kit (Qiagen). Samples were then sent to the University of Minnesota Genomics Center for library preparation and 16S rRNA gene and ITS sequencing on 2 x 300 bp PE MiSeq flow cell. Demultiplexed FASTQ files were returned and the quality of the paired end reads was checked using fastp (v.23.2) (Chen et al., 2018). After quality control, reads were preprocessed using the QIIME 2 (v2023.2) (Bolyen et al., 2019) pipeline. Briefly the DADA2 (v2022.2.0) (Callahan et al., 2016) algorithm was used to further filter, trim and denoise the paired end reads to obtain amplicon sequence variants (ASV) and a feature table. Taxonomy of sequences were inferred using naïve-bayes sequence classifiers. For

16S rRNA data classifier trained on SILVA138_AB_V4 database (Quast et al., 2012) was used. For ITS data classifier trained on UNITE v9.0 (Põlme et al., 2020) was used. QIIME2 functions were used to determine Alpha group diversity, Beta group diversity and Principal Coordinates. The Kruskal-Wallis test implement in ALDEx2, R-package (v1.33.0) (Fernandes et al., 2013) to test for differential abundance of taxa in a pairwise manner.

Hemp Yield and Cannabinoid Content

At seven weeks after flowering the top 5-8 inches from the primary inflorescence of two plants in each treatment was sampled and sent to Rock River Laboratory (Watertown, WI) for cannabinoid analysis using High Performance Liquid Chromatography (HPLC). Total THC and CBD were calculated from this analysis using the following formulas: Total CBD = cannabidiol (CBD) + (cannabidiolic acid (CBDA)*0.877) and Total THC = delta-9-tetrahydrocannabinol (Δ9-THC) + (tetrahydrocannabinolic acid (THCA)*0.877). Hemp plants were harvested 10/9/21 in Hayward, 10/22/21 in West Madison, and 10/14/22 in West Madison the second year. At harvest, height was collected by measuring from the base of the plant to the top most meristem with a meterstick (cm). Two plants from each treatment per block were placed in drying ovens (120°F), and weighed after reaching a constant temperature. Dried floral mass was removed from one plant and weighed to measure biomass yield.

All data can be found in Appendix A supplementary Table 1.

Data Analysis

Data analysis was performed in RStudio version 2022.12.0 using R statistical software version 4.2.2 (R Core Team 2022). For each trait, data assumptions of normality and homogeneity of variance were assessed using QQ and residuals vs. fitted plots. When normality was not met, data were square root transformed. This transformation was done to the following insect samples:

beneficial sample 2, pest sample 2, and incidental sample 2. A mixed-model analysis of variance (ANOVA) was selected to analyze this experiment with year and companion treated as fixed effects and the effect of block nested within year defined as random. R package “lme4” was used for mixed effects modeling (Bates et al., 2015). Pairwise means were compared with Tukey’s Honest Significant Difference using the function “emmeans”, with significance accepted at $\alpha < 0.05$. Results were visualized using the R package “ggplot2” (Wickham, 2011).

Results

Weed Cover

Weed cover ranged from 0 to 85 percent between companions across all locations. A significant difference was found between companions and their ability to suppress weeds, ($p < 0.001$) (Table 1). The marigold treatment decreased weed cover when compared to all other treatments ($p<0.05$ for marigold compared to all treatments). Basil also significantly decreased weed cover ($p= 0.032$) when compared to blank treatments, appearing to have a suppressive effect on weeds across all environments. All other treatments -dill, blank, cilantro, and sage -did not statistically differ from each other in their ability to suppress weeds (Figure 4). There was a strong environment effect on weed cover ($p < 0.001$), but no companion by environment interaction ($p = 0.873$) (Table 1).

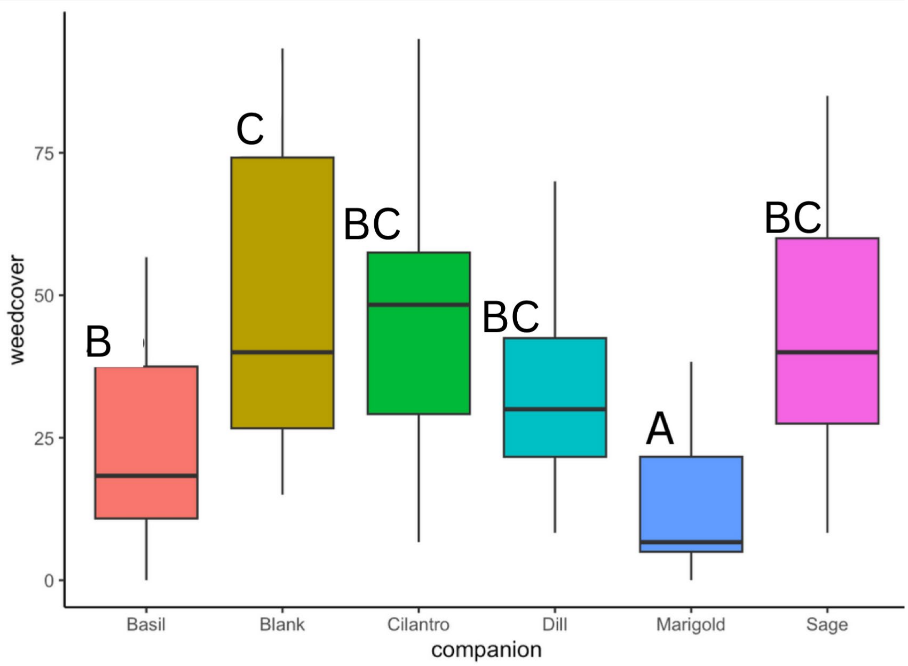


Figure 1: Boxplot depicting the percent weed cover (%) by companion treatment. Different letters represent statistical differences as determined by HSD means separation.

Table 1: ANOVA depicting the F and P-values for weed cover. Significant interactions highlighted. Environment is the combination of the three replications of experiment.

Treatment Effects	DF	F value	P-Value
Companion	5	6.4232	0.0001815
Environment	2	14.4007	1.95E-05
Environment:Block	8	1.4908	0.1912515
Companion:Environment	10	0.8733	0.5648232

Plant Height

Plant height ranged from 87.2 cm to 175.8 cm between companions across environments. No significant difference was found between companions and hemp plant height ($p=0.26$). While there was no interaction between companion and environment ($p=0.834$), there was a strong environment effect on plant height ($p<0.001$) (Table 2).

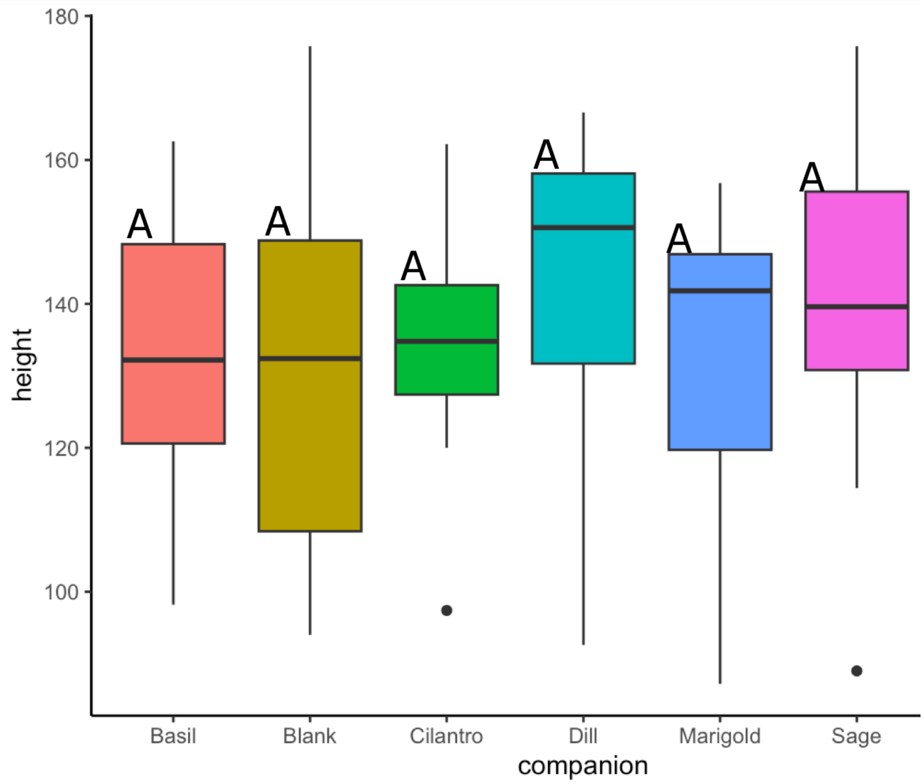


Figure 2: Boxplot depicting hemp height (cm) by companion treatment. Different letters represent statistical differences as determined by HSD means separation.

Table 2: ANOVA depicting the F and P-values for hemp plant height. Significant interactions highlighted. Environment is the combination of the three replications of experiment.

Treatment Effects	Df	F Value	P-Value
Companion	5	187.78	0.26
Environment	2	44	9.05e-06
Environment:block	8	0.835	9.32e-06
Companion:Environment	10	1.0461	0.8338

Dry and Bucked Weight

Dry weight ranged from 0.2 to 2.56 pounds across companions and environments. Companion treatments did not statistically impact the dry weight of hemp plants, but there was a difference between environments for dry weight ($p<0.001$). There is no interaction between environment and block, or companion by environment (Table 3). Bucked weight ranged from 0.015 to 1.35 pounds across environments and companions. There was no statistical difference between companion treatments and bucked weight of hemp. There is a statistical difference between bucked weights and environment ($p<0.001$), and an interaction was observed between blocks nested in environment with block 4 in year 2 producing significantly smaller plants. No other significant interactions were observed between companions and environments (Table 3).

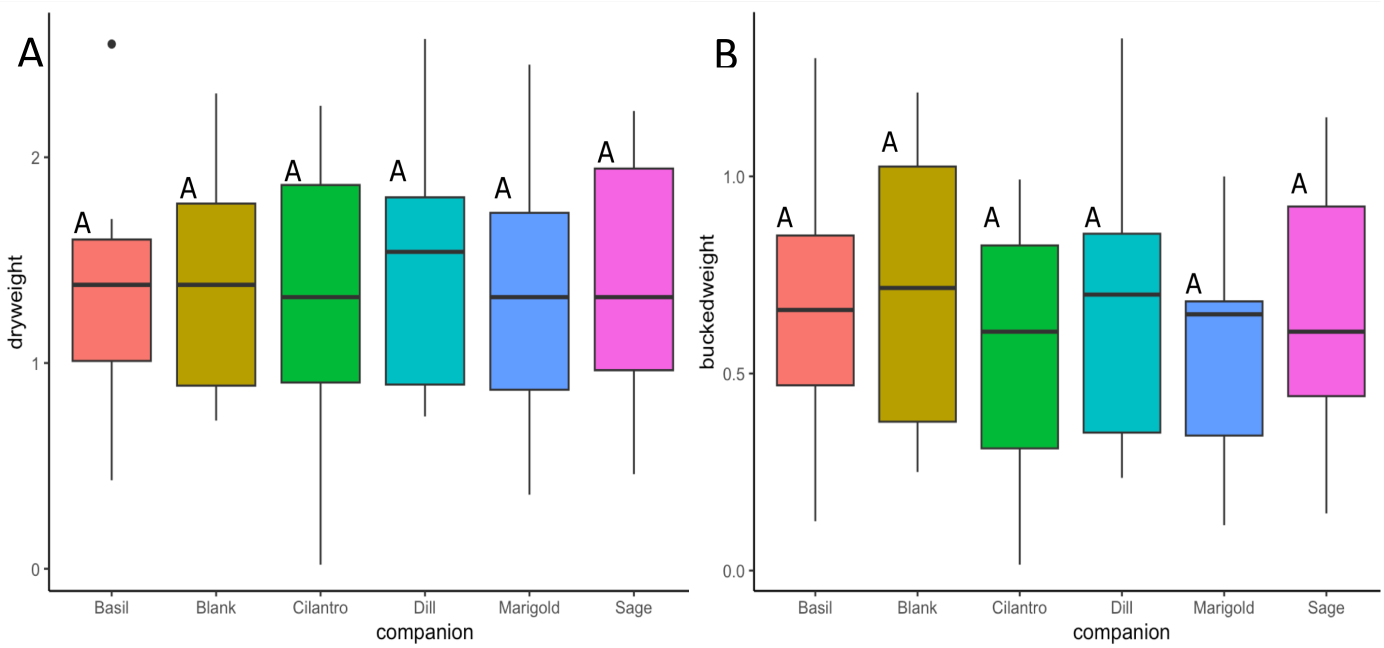


Figure 3: Boxplot depicting the (A) dry weight and (B) bucked weight of hemp (lbs) by companion treatment. Different letters represent statistical differences as determined by HSD means separation.

Table 3: ANOVA depicting the F and P-values for dry and bucked weight. Significant interactions highlighted. Environment is the combination of the three replications of experiment.

		Dry Weight		Bucked Weight	
Treatment Effects	DF	F value	P-Value	F value	P-Value
Companion	5	0.2172	0.9531	1.0604	0.3966
Environment	2	29.7379	1.22E-08	45.1297	5.56E-11
Environment:Block	8	1.408	2.23E-01	0.6718	7.30E-01
Companion:Environment	10	0.4482	0.9127	0.3937	0.9418

Companion Harvest Price

Companion harvest potential value ranged from 0 to 798 dollars between companion treatments across all environments. A significant difference was found between companions and their possible value, ($p<0.001$) (Table 4). The basil treatment had significantly more potential value than any other treatment ($p<0.001$). Cilantro, dill, and marigold had significantly more possible worth than blank and sage, which were significantly similar and had the least potential value. There was a strong companion by environment interaction on price ($p<0.001$), so environments were analyzed separately (Figure 4).

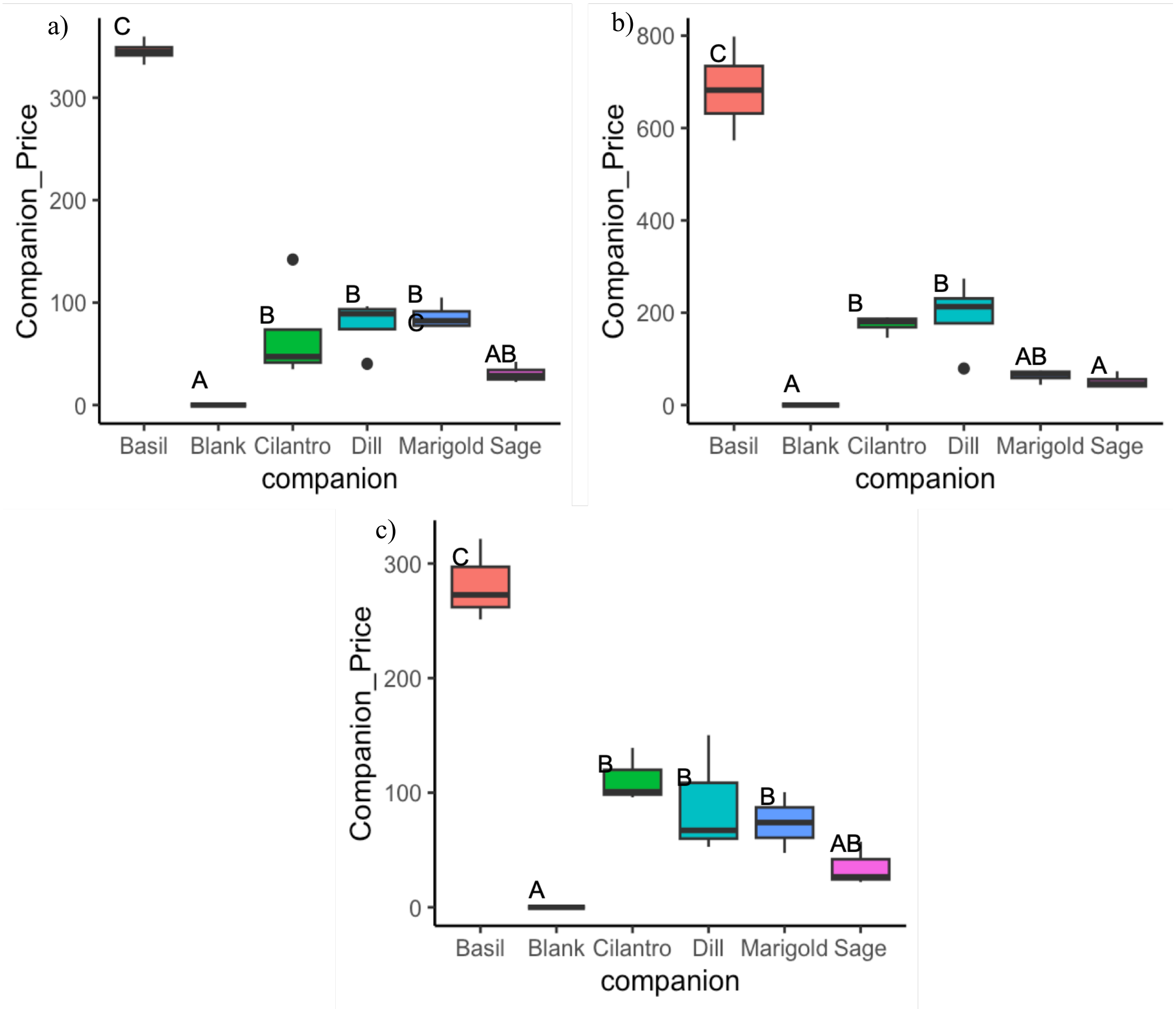


Figure 4: Boxplot depicting the price of companion harvest ($) by companion treatment for (a) environment 1, (b) environment 2, and (c) environment 3. Different letters represent statistical differences as determined by HSD means separation.

Table 4: ANOVA depicting the F and P-values for companion harvest value for environments 1, 2, and 3. Significant interactions highlighted.

	ENV1 Cost of Companion			**Env2 Cost of Companion**		**Env3 Cost of Companions**		
Treatment Effects	DF	F value	P-Value	F value	P-Value	DF	F value	P-Value
Companion	5	101.8523	4.97E-11	82.7441	2.23E-10	5	42.599	2.01E-06
Block	3	0.8651	4.81E-01	0.4825	6.99E-01	2	3.409	7.43E-02

Cannabinoid Content

THC ranged from 0.07 to 0.525 percent between locations and treatments; CBD ranged from 2.91 to 14.76 percent between locations and treatments. Both THC and CBD were not statistically impacted by companion treatments (Figure 5). There was a statistical difference between environments and blocks nested within environments (p-value<0.001) (Table 5). There was no environment interaction with companion (Table 5).

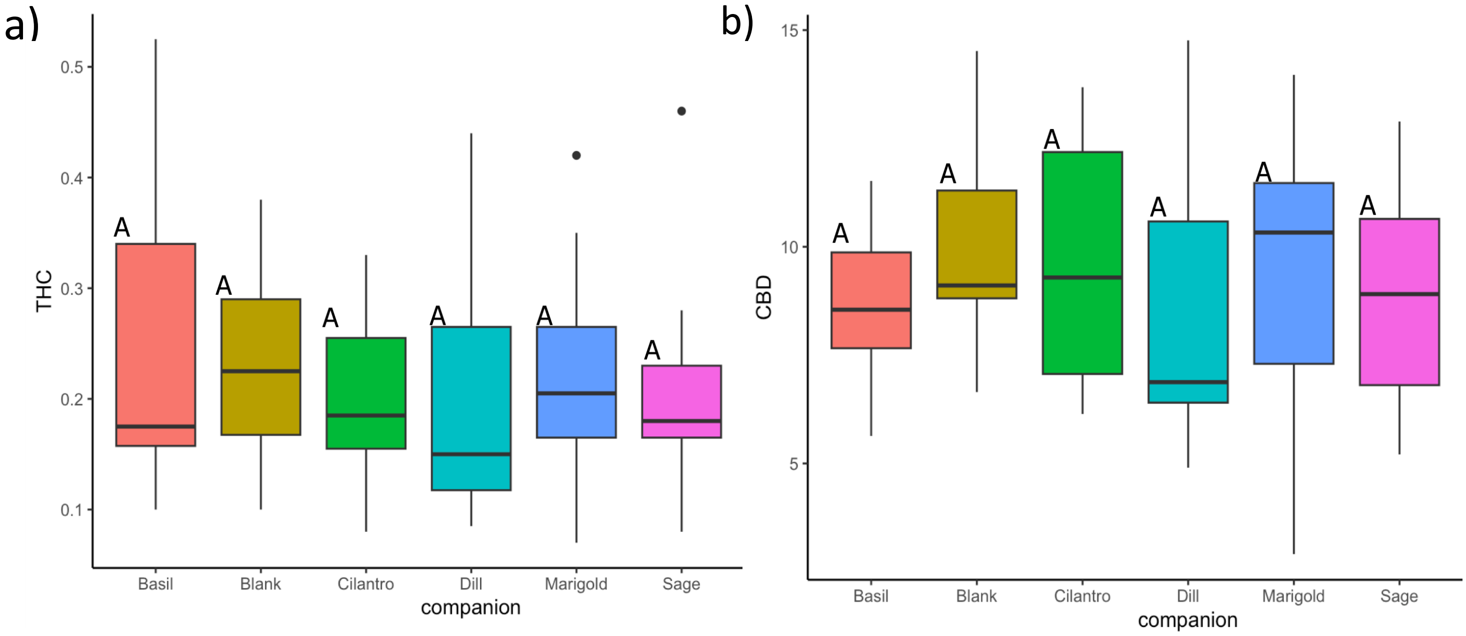


Figure 5: a) Boxplot depicting the CBD content (% dry weight) by companion treatment. b) Boxplot depicting THC content (% dry weight) by companion treatment. Different letters represent statistical differences as determined by HSD means separation.

Table 5: ANOVA depicting the F and P-values for CBD and THC percent. Significant interactions highlighted. Environment is the combination of the three replications of experiment.

		CBD		**THC**	
Treatment Effects	DF	F value	P-Value	F value	P-Value
Companion	5	0.8419	0.5281	0.7561	0.5867
Environment	2	41.3392	1.84E-10	28.4588	2.06E-08
Environment:Block	8	0.9554	4.84E-01	0.835	5.78E-01
Companion:Environment	10	1.3482	0.2394	1.0461	0.4246

Insect Composition

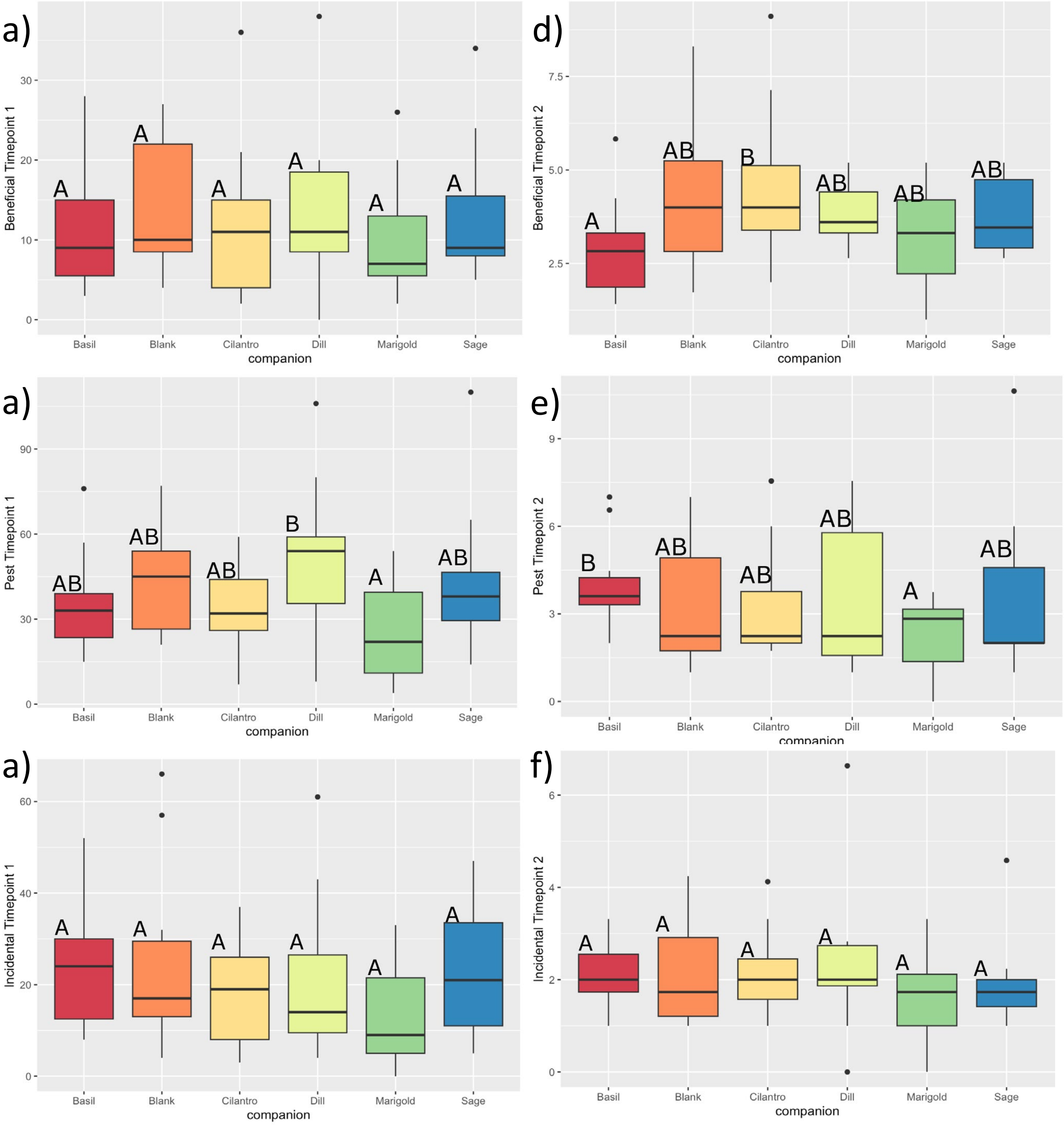


Figure 6: Boxplot depicting insects in (a) beneficial timepoint 1, (b) pest timepoint 1, (c) incidental timepoint 1, (d) beneficial timepoint 2, (e) pest timepoint 2, and (f) incidental timepoint 2. Different letters represent statistical differences as determined by HSD means separation. All categories in timepoint 2 have been transformed by square root to fit normality.

Table 6: ANOVA depicting the F and P-values for all insect categories for both timepoints. Significant interactions highlighted. Environment is the combination of the three replications of experiment.

	Beneficial 1			Pest 1		Incidental 1	
	DF	F Value	P-Value	F Value	P-Value	F Value	P-Value
Companion	5	0.837	0.531	2.47	0.048	2.215	0.072
Environment	2	21.243	5.17E-07	8.44	0.00087	30.192	1.02E-08
Environment:Block	8	3.039	0.0091	1.81	0.104	3.453	0.004
Companion:Environment	10	1.691	0.117	0.21	0.994	1.908	0.072
	Beneficial 1			Pest 2		Incidental 2	
Companion	5	2.583	0.041	2.72	0.033	0.871	0.509
Environment	2	7.783	0.0014	66.19	2.05E-13	6.197	0.005
Environment:Block	8	1.039	0.424	2.54	0.025	2.001	0.071
Companion:Environment	10	1.414	0.209	1.95	0.066	0.645	0.767

There were more total insects in timepoint one than timepoint two across all classifications (pest, beneficial, incidental). At the first timepoint sampling, significant differences were found for the number of pests between treatments specifically between marigold and dill, with marigold having statistically less pests present that dill in all three environments (Figure 7b, Table 6). No statistical differences were observed for the other categories in timepoint one, meaning that there were no differences between numbers of beneficial or incidental insects between companion crops. Statistical differences were found between environments in all categories– beneficial, incidental, pest— for the first timepoint ($p < 0.001$) but there was not any interaction between companion and environment ($p > 0.1$).

Insect results from timepoint two highlight a significant difference between companions ($p=0.041$) specifically between basil and cilantro treatments, with cilantro having more beneficial insects than basil (Figure 6d). When looking at pests in this timepoint, there was a statistical difference between basil and marigold, with marigold having less pests than basil (Figure 6e). There were no statistical differences between companion crops with incidental insects (Figure 6f). There were again, statistical differences between all environments with each category in the second timepoint (Appendix B), but no interaction between environment and companion crop so environments were analyzed together. The most common pest, beneficial and incidental insect populations across all categories are highlighted in Figure 7.

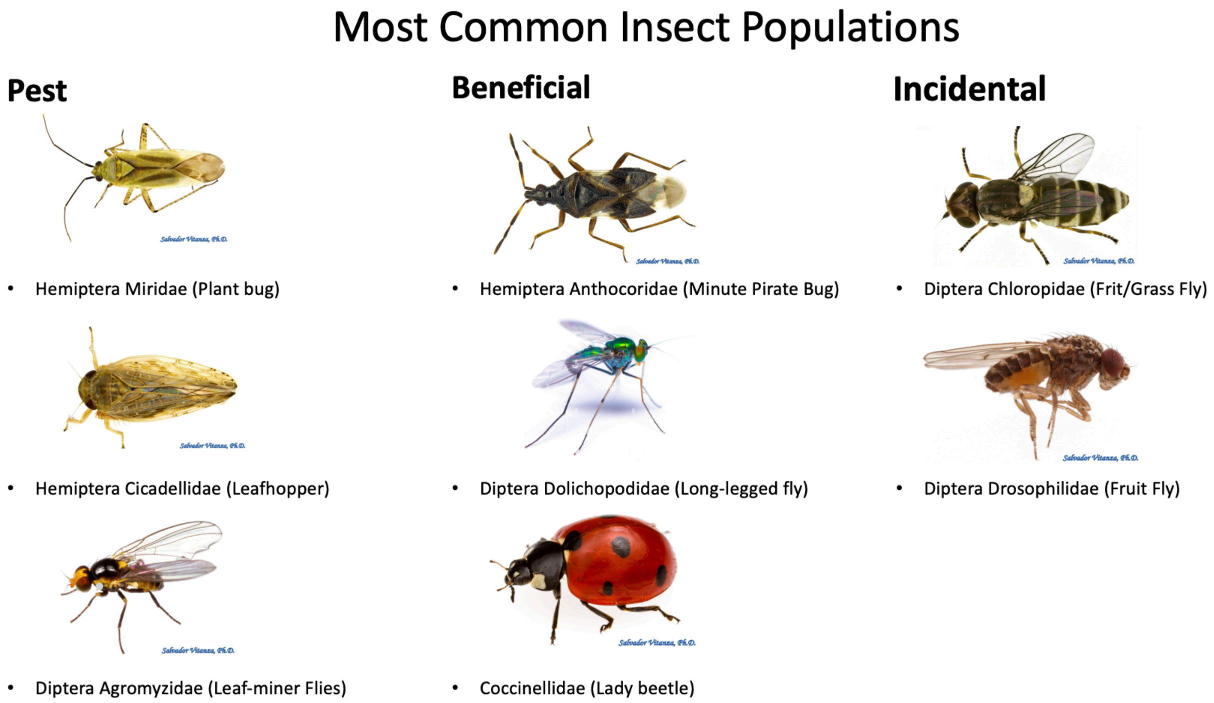


Figure 7: Most common insect populations that were observed across all environments and timepoints. Raw data available upon request.

Microbiome Diversity

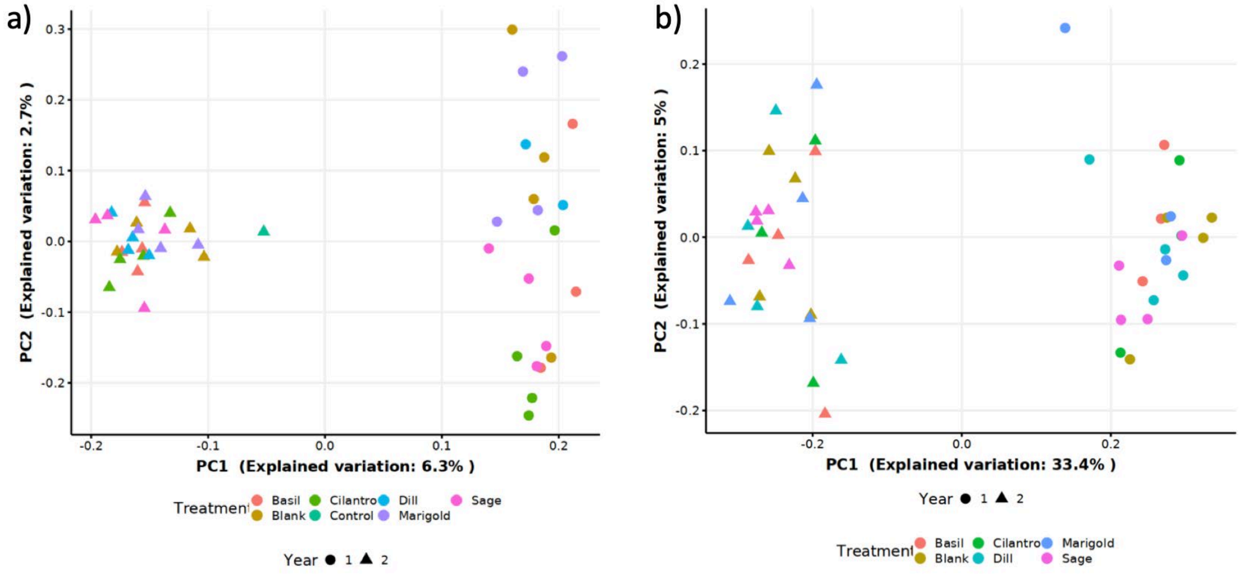


Figure 8: Principal Coordinate analysis for (a) 16S and (b) ITS.

Principal Coordinate analysis (PCoA) was conducted to identify clustering between years and companion treatments. There is a clear distinction between years, with PC1 aligning with year and explaining 33.4% of the variation for ITS and 6.3% of variation for 16S (Figure 8). However, within years, treatments show no clear clustering, meaning that there is no distinction between treatments and the microbial species that are present (Figure 8). This clear year distinction and no treatment distinction is seen with both the 16S and ITS.

A Shannon diversity index (SDI) was used to measure bacterial and fungal diversity. SDI were between 6.2 and 7.5 for 16S (bacteria) and 7.2 and 9.5 for ITS (fungi). No significant differences were found between companion treatments for either 16S or ITS results (Figure 9), but there were large differences between years (Figure 10). There were also significant differences between years and the number of species present ($p<0.05$)(Appendix A). Raw microbiome data available upon request.

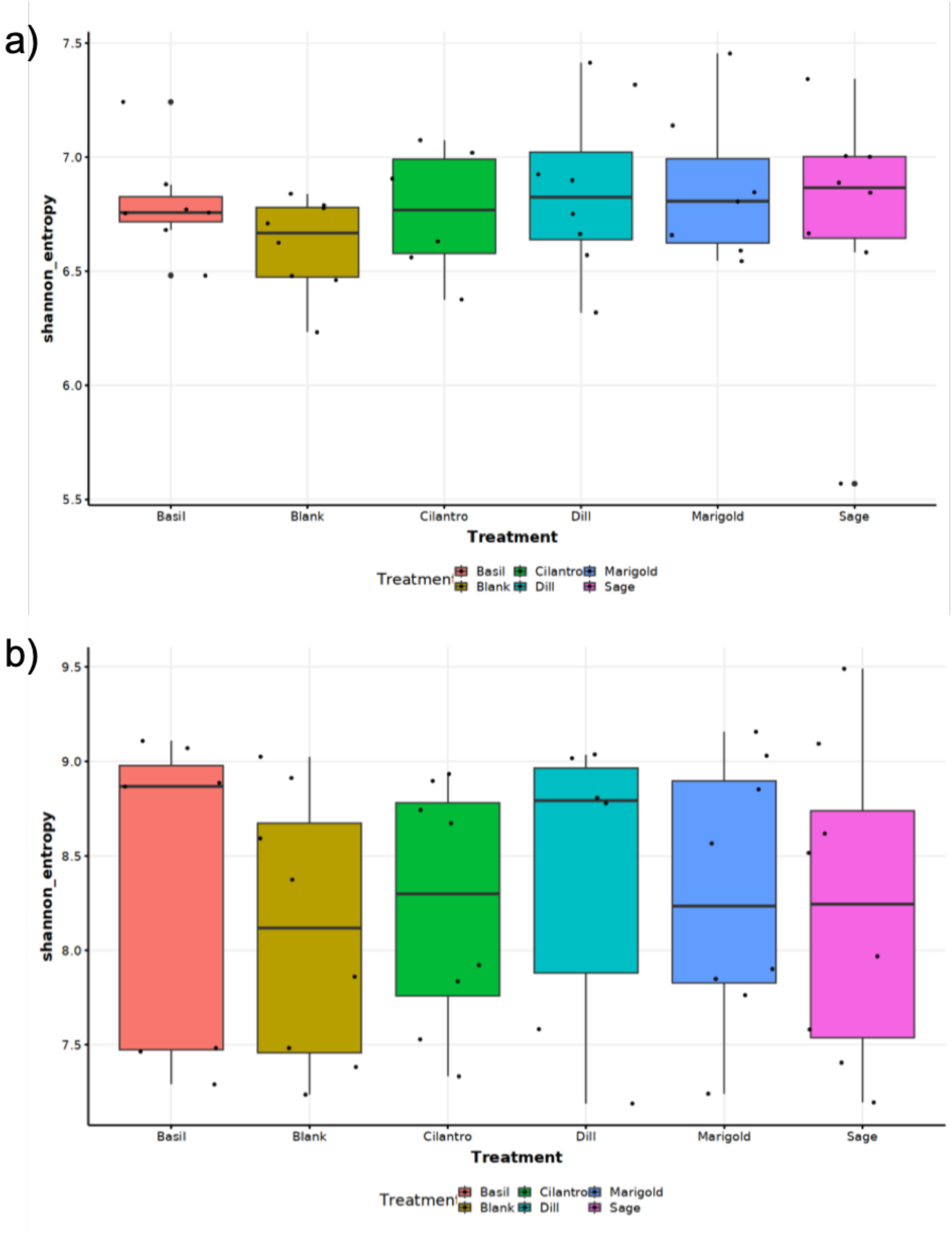


Figure 9: Shannon diversity index for (a) 16S and (b) ITS. No statistical difference was found between treatments.

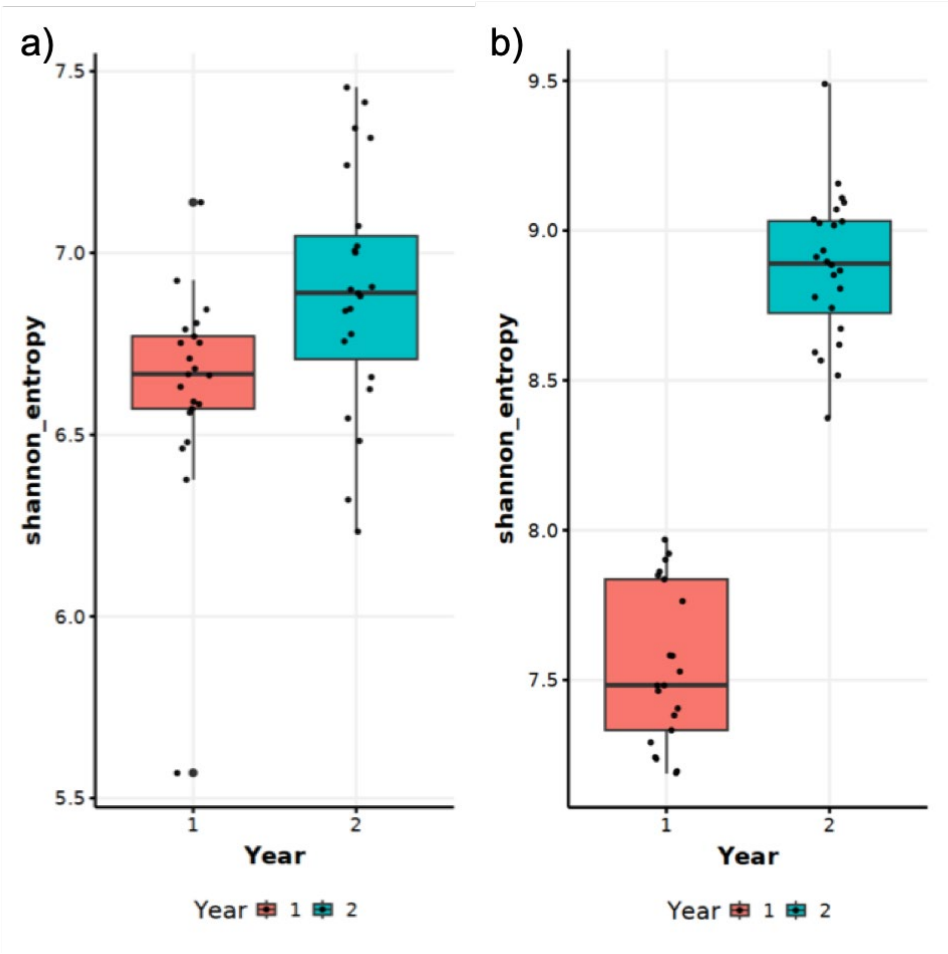


Figure 10: Shannon diversity between years for (a) 16S and (b) ITS.

Discussion

Due to increased climatic pressures, higher input costs, and ecosystem degradation, it is essential to explore alternative cropping systems that are resilient, sustainable, and productive. With the recent relegalization of hemp there is an opportunity to explore alternative cropping systems, such as intercropping, that might better fit the tenets of agroecology. While intercropping has been utilized in many cultures throughout history, research documenting the ecosystem services provided by this cropping system are rare. The work presented here will establish agroecological benefits provided by the successful adoption of companion cropping in cannabis.

Weed Cover and Erosion Control

Our results reinforce the idea that companion cropping can suppress weeds without the addition of herbicides (Yeganehpoor et al., 2015). With current regulations in hemp, no synthetically derived herbicides are allowed for use in hemp production systems (EPA, 2023). This highlights the importance of integrated weed management not only for organic producers, but also conventional growers who have no options for chemical use. Integrated weed management (IWM) is a component of integrated pest management (IPM), that focuses on dealing with weeds in production settings holistically, considering ways to reduce weed communities instead of weed species (Riemens et al., 2022).

This idea of IWM highlights the potential importance of diversified cropping systems in cannabis. With increased negative impacts of herbicides on human health, biodiversity, and waterways, developing systems that have a decreased need for these external inputs is necessary (Riemens et al., 2008, Storkey et al., 2012; Kreuger, 1998). The companions, marigold and basil had the largest decrease in weed cover (Figure 4b) suggesting these two crops have good potential to compete with early establishing weeds in a hemp production system.

Along with decreased weed cover, these companions also have the ability to aid with erosion control. Conventional agricultural field management can drastically erode topsoil— a nonrenewable resource in this lifetime— with increased tillage and bare ground, exacerbated by severe water events (Pimentel & Burgess, 2013). Current high cannabinoid hemp production can leave up to two meters between rows, with up to 50% of the bare ground exposed during the first six weeks of production. Companion cropping decreases erosion by covering more ground and establishing roots to better hold soil in place, with a recent study showing that soil loss decreased 26-43% by intercropping (Ahmad et al., 2020). This is not only very important for the future of cannabis production, but agriculture in general. Identifying best practices that make cropping systems resilient to increased climatic pressures such as erosion from increased extreme weather events, is essential for a sustainable agricultural future. Basil and marigold were able to cover the alleyways between hemp plants, leaving almost no bare soil that could lead to erosion during rain events. This is in contrast with the blank treatment, which mimicked a typical monocropped field, where the soil was left bare, leaving many opportunities for erosion with heavy rain events (Picture 2).

Picture 2: Blank, marigold, and basil treatments year one at WMARS three weeks into growing season. Basil and marigold treatments visually have fewer weeds in alleyways as compared to the blank treatment

Yield: Cannabinoids, Companion Crops, Biomass

When establishing best practices, one of the most important factors for growers is yield, especially making sure that yield does not decrease. For hemp growers, another key aspect is that cannabinoid content is not altered, as yield and price directly relate to cannabinoid abundance. Cannabidiol (CBD) content was not affected by companion crops. Cannabinoids are one type of secondary metabolites which accumulate in the female flower of hemp (Jin et al., 2020), and are what is harvested from high cannabinoid hemp production sites. When comparing all companion treatments to the blank treatment, there were no statistical differences in CBD content. This is important, as it ensures that none of the companions chosen in our study produce secondary metabolites that could alter the production of cannabinoids in hemp. With no alteration of cannabinoids, growers can be sure that they can plant any of these companion plants without having to worry about the makeup of their plants.

There were larger variations observed in yield and cannabinoid content overall, with plant height ranging from 87.2 to 175.8 cm, weight from 0.2 to 2.56 pounds, and CBD percent from 2.91 to 14.6. This is likely due to the genetic variability in the cherry wine cultivar used, as well as the state of hemp cultivars in general at the moment. It would be interesting to repeat this study once cultivars become more uniform to allow for better observations in small differences there may be between companions and their effect on hemp yield.

Another cannabinoid that can drastically affect a grower's income is Tetrahydrocannabinol (THC). There is a strict 0.3% THC limit for hemp compliancy in the U.S. (USDA, 2022). If a field is tested and exceeds this 0.3% cutoff it has to be destroyed. For a grower, this means that their entire income from hemp will be lost. To ensure compliancy in a companion cropping system, THC cannot statistically differ between different companion treatments, or exceed the 0.3% threshold. Fortunately, there was no interaction between companion type and THC content, implying that a grower does not have to worry about noncompliance when choosing a companion crop.

There was no statistical difference between companion crop treatments and hemp height, dry weight, or bucked weight. This is a very important finding as it means that the most prolific companion crops, basil and marigold, do not negatively impact, or compete, with hemp for resources. For many growers, outside inputs are necessary to ensure high crop yields. This study received no outside inputs—fertilizers or irrigation—and we observed no competition between companion crops with hemp. This information is essential, as it shows that even in a low input system, these crops are not competing for nutrients. Under a typical growing system where fertilizer and irrigation are applied, this would be even less concerning as there would be more available nutrients that are essential for growth.

There was a statistical difference between companion crops and their possible contribution as a source of secondary income, with basil statistically more valuable than all other companions. This stark difference most likely is attributable to being able to harvest basil multiple times throughout the growing season; whereas, the other crops were only harvested once. This extra harvest allowed for more yield, and therefore more income. Multiple plantings would have to happen for the other companion crops to be as profitable as basil.

Beneficial, Pest, and Incidental Insect Abundance

The results of our insect diversity assessment highlight a possible difference in companion crops and the species that are on or surrounding them. Specifically, we saw that marigold had statistically less pests in both timepoints than all other companion crops. How insects choose which plant they visit is dependent on a combination of factors including chemical, visual, and genetic factors (Parker et al., 2013). The selected companions in this study are known to have strong odors associated with secondary metabolites, pigments, and inflorescence architecture that has been used to attract or deter certain groups of insects. Specifically, one study found that when planting marigolds alongside tomatoes, the number of glasshouse whitefly eggs decreased significantly, most likely due to repellent volatile chemicals from marigolds (Stratton et al., 2022). Another study found that the presences of basil was able to increase the health and offspring of the lacewing, a natural enemy of aphids. This increase in lacewing ultimately has the ability to act as biological control on aphids, with basil acting as a functional plant aiding in the amount of lacewings present (Fang et al., 2022). Observing differences in our study reinforces the idea that even in a small space, insect diversity can vary greatly. The data also strengthens the idea that when companion crops are present, there will be less pests on the main crop; this is because of volatile compounds from companion crops, that can repel pests and throw them of off the main crop, as well as pests being lured to companion crops (Peter et al., 2023; Finch & Collier, 2011). Despite large numbers of insects collected in our sweeps, there was very little visible insect damage on the hemp across all environments, and no damage that would impact yield. A follow-up study to characterize the association of secondary metabolites with insect predation may help inform appropriate companions to deter specific insect pests.

Climate change can cause insect populations to shift and/or lead to increases in pest species, causing a need for additional pesticide applications (Altieri et al., 2015). If a crop such as dill or basil, that had significantly more pest pressure than the other crops—and significantly less beneficial insects in the case of basil—, was all that was in the field, there would possibly be an increased amount of damage from pests, as well as a need for pesticides. Because there was such a large amount of diversity in crop species in this project, one crop having more pests did not result in the need for any outside inputs. It also did not result in the main crop, hemp, being damaged at all. This is paramount, as it highlights the importance of diversity in agriculture, leading to more resilient cropping systems. It will be important to see if intercropping with a single species, thereby reducing crop diversity, has a similar affect.

Microbiome

The microbiome results from this study indicate no differences in bacterial or fungal species abundance between companion treatments. There was however, a very significant difference between years. It is likely that differences between companions were not observed for several reasons. Namely, the length of the experiment and experimental design. If we had planted this experiment in the same plot of land with the same design for two years we may have seen differences between species composition and abundance. We also may have seen differences if the hemp plants and companions were planted closer together. Due to our sampling methods, we sampled close to the hemp plant early in the season, so sampling closer to companions later in the season may have shown a different interaction between microbes and companion/hemp combinations. Regarding differences between years, we hypothesize this was caused mostly from changes in plot location. Soils are diverse and extremely heterogeneous, meaning that even across short distances, there may be differences in microbial composition (Kuzyakov et al., 2015). While in both years the West Madison Agricultural Research Farm was used, our experiment was planted on different plots approximately 500 meters apart. These sites likely had different conditioning which could lead to large changes in soil dynamics and makeup. Additionally, climatic differences in temperature and rainfall between years may also contribute to observed variation.

Our current data suggest that companion crops will not alter the microbiome within hemp crops, implying a grower could choose any of the companion crops tested and not have to worry about them altering their microbial community. It also means that there could be growers on different sides of the state or even the U.S. with completely different soil types, and they may be able to follow the same guidelines without worry of hemp and a companion negatively impacting microbial composition. As this experiment was limited in time and scope we do suggest follow-up experiments be conducted before making broad generalizations.

Alternative cropping systems and agroecology are social movements; a way for people to organize and take charge of their community and environment and create positive change in U.S. agricultural systems. Companion cropping is well aligned with agroecology’s holistic approach. Economic sovereignty by way of increased yield/income coupled with increased resiliency and ecosystem health is key for growers. The transdisciplinary approach that is agroecology has the capacity to transform hemp’s best production practices into something that benefits all involved, moving beyond a strict monocropping approach, and into a holistic one that takes all aspects and players of production into account.

Future Directions

In order for companion cropping to become a more practiced growing method in cannabis, on farm trials are necessary. These trials need to consist of larger plot sizes to better understand the limitations of companion cropping at scale. They also need to utilize standard cultivation practices, with equipment that growers already own. It is essential that companion cropping works within the confines of what resources are available to growers, or else they may be less likely to adopt the practice. By having on farm trials, spacing of companions can be better understood and layouts can be changed based off of growers needs: this is an integral next step as it puts companion cropping in cannabis on a larger scale where real life challenges growers face can be examined. It is also the only way to monitor how ecosystem services are being preserved, and how they are helping out with a limited input growing system while still allowing for high yields. On farm trials are also necessary considering environment was statistically different for every trait studied, which shows that environmental factors such as soil type, rain fall, and temperature have a great impact on ecosystem services.

Cover cropping is another popular agroecological cropping system that is being used by farmers to preserve ecosystem services (Silva & Moore, 2017). The difference between cover cropping and companion cropping is that cover crops are not commonly harvested and sold for profit and are typically grown in rotation with other crops (Stratton et al., 2022); whereas, companion crops are grown alongside other crops and typically harvested to be sold. These two agroecological cropping systems could be compared to see which works best with hemp cultivation. There are a plethora of ways that this could work, but there could be in row cover cropping with a nitrogen fixing legume, or there could be a clover cover crop in between growing seasons in a rotation. This could then be compared to this companion cropping research to see which is more fit for hemp production.

To better understand the effect of companion crops on soil microbiome composition and soil health with regards to ecosystem services, it would be beneficial to have a long-term companion cropping soil health assessment and include measurements such as carbon sequestration and nitrogen levels, along with microbial assessments. This would allow a better understanding of how companion cropping impacts soil dynamics over time. It would also allow for more specific microbial species and their abundance to be studied, better understanding species composition and corresponding functions.

Lastly, it will be important to develop hemp cultivars for intercropping systems that take a systems approach, not a monocropping approach. In order to make agroecological cropping systems such as companion cropping work, there needs to be thought put into the main crop as well as the companion crop. Breeding for resilient and sustainable crops needs to be a top priority to ensure a future with food security and ecological health, especially in the face of climate change.

References

Ahamad, N.S.B.N., Mustaga, F.B., & Didams, G. (2020). A systemic review of soil erosion control practices on the agricultural land in Asia. International Soil and Water Conservations Research, 8(2), 103-115.

Altieri, M. A., Nicholls, C. I., Henao, A., & Lana, M. A. (2015). Agroecology and the design of climate change-resilient farming systems. *Agronomy for Sustainable Development*, *35*(3), 869–890. https://doi.org/10.1007/s13593-015-0285-2

Bogužas, V., Skinulienė, L., Butkevičienė, L. M., Steponavičienė, V., Petrauskas, E., & Maršalkienė, N. (2022). The Effect of Monoculture, Crop Rotation Combinations, and Continuous Bare Fallow on Soil CO2 Emissions, Earthworms, and Productivity of Winter Rye after a 50-Year Period. *Plants*, *11*(3), 431. https://doi.org/10.3390/plants11030431

Brooker, R. W., Bennett, A. E., Cong, W., Daniell, T. J., George, T. S., Hallett, P. D., Hawes, C., Iannetta, P. P. M., Jones, H. G., Karley, A. J., Li, L., McKenzie, B. M., Pakeman, R. J., Paterson, E., Schöb, C., Shen, J., Squire, G., Watson, C. A., Zhang, C., … White, P. J. (2015). Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. *New Phytologist*, *206*(1), 107–117. https://doi.org/10.1111/nph.13132.

Brym, Z. T., & Reeve, J. R. (2016). *Agroecological Principles from a Bibliographic Analysis of the Term Agroecology* (pp. 203–231). https://doi.org/10.1007/978-3-319-26777-7_5

Cardinale, B. J., Wright, J. P., Cadotte, M. W., Carroll, I. T., Hector, A., Srivastava, D. S., Loreau, M., & Weis, J. J. (2007). Impacts of plant diversity on biomass production increase through time because of species complementarity. *Proceedings of the National Academy of Sciences*, *104*(46), 18123–18128. https://doi.org/10.1073/pnas.0709069104

Cruz, J. C., House, L. A., & Blare, T. D. (2021). Global overview of hemp production and the market of hemp-derived CBD in the US. 2021 Annual Meeting, August 1-3, Austin, Texas 31297, Agricultural and Applied Economics Association. DOI: 10.22004/ag.econ.312927

EPA, 2023. https://www.epa.gov/pesticide-registration/pesticide-products-registered-use-hemp

Fang, Y., Li, S., Xu, Q., Wang, J., Yang, Y., Mi, Y., Jin, Z., Desneux, N., & Wang, S. (2022). Optimizing the Use of Basil as a Functional Plant for the Biological Control of Aphids by Chrysopa pallens (Neuroptera: Chrysopidae) in Greenhouses. *Insects*, *13*(6), 552. https://doi.org/10.3390/insects13060552

Finch, S., & Collier, R. H. (2012). The influence of host and non-host companion plants on the behaviour of pest insects in field crops. *Entomologia Experimentalis et Applicata*, *142*(2), 87–96. https://doi.org/10.1111/j.1570-7458.2011.01191.x

Griffiths-Lee, J., Nicholls, E., & Goulson, D. (2020). Companion planting to attract pollinators increases the yield and quality of strawberry fruit in gardens and allotments. *Ecological Entomology*, *45*(5), 1025–1034. https://doi.org/10.1111/een.12880

Harris, N., & Streets, J. (2022). *Companion planting*. Extension. https://extension.wvu.edu/lawn-gardening-pests/gardening/garden-management/companion-planting#:~:text=Companion%20planting%20allows%20you%20to,deeper%20in%20the%20soil%20profile.

Jin, D., Dai, K., Xie, Z., & Chen, J. (2020). Secondary Metabolites Profiled in Cannabis Inflorescences, Leaves, Stem Barks, and Roots for Medicinal Purposes. *Scientific Reports*, *10*(1), 3309. https://doi.org/10.1038/s41598-020-60172-6

Kreuger, J. (1998). Pesticides in stream water within an agricultural catchment in southern Sweden, 1990–1996. *Science of The Total Environment*, *216*(3), 227–251. https://doi.org/10.1016/S0048-9697(98)00155-7

Kuzyakov, Y., & Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil: Concept & review. *Soil Biology and Biochemistry*, *83*, 184–199. https://doi.org/10.1016/j.soilbio.2015.01.025

Lan, Y., Zhang, H., He, Y., Jiang, C., Yang, M., & Ye, S. (2023). Legume-bacteria-soil interaction networks linked to improved plant productivity and soil fertility in intercropping systems. *Industrial Crops and Products*, *196*, 116504. https://doi.org/10.1016/j.indcrop.2023.116504

Mangan, A. (n.d). Decolonizing Regenerative Agriculture: An Indigenous Perspective. *Bioneers*.

Marzani, Q. A. (2023). Management of ascochyta blight (Ascochyta rabiei (Pass.) Labrouse) using chickpea-based mixed cropping systems through different combinations of companion crops. *Australasian Plant Pathology*, *52*(3), 215–223. https://doi.org/10.1007/s13313-023-00912-0

Mehrabi, Z., Delzeit, R., Ignaciuk, A., Levers, C., Braich, G., Bajaj, K., Amo-Aidoo, A., Anderson, W., Balgah, R. A., Benton, T. G., Chari, M. M., Ellis, E. C., Gahi, N. Z., Gaupp, F., Garibaldi, L. A., Gerber, J. S., Godde, C. M., Grass, I., Heimann, T., … You, L. (2022). Research priorities for global food security under extreme events. *One Earth*, *5*(7), 756–766. https://doi.org/10.1016/j.oneear.2022.06.008

Nasir Ahmad, N. S. B., Mustafa, F. B., Muhammad Yusoff, S. @ Y., & Didams, G. (2020). A systematic review of soil erosion control practices on the agricultural land in Asia. *International Soil and Water Conservation Research*, *8*(2), 103–115. https://doi.org/10.1016/j.iswcr.2020.04.001

Parker E., J., E., W., C., G., & RodriguezSao, C. (2013). Companion Planting and Insect Pest Control. In *Weed and Pest Control - Conventional and New Challenges*. InTech. https://doi.org/10.5772/55044

Peter, E., Tamiru, A., Sevgan, S., Dubois, T., Kelemu, S., Kruger, K., Torto, B., Yusuf, A. (2023). Companion crops alter olfactory responses of the fall armyworm (Spodoptera frugiperda), and its larval endoparasitoid (Cotesia icipe). *Europe PMC.* DOI: 10.21203/rs.3.rs-2535302/v1

Philip Robertson, G., Gross, K. L., Hamilton, S. K., Landis, D. A., Schmidt, T. M., Snapp, S. S., & Swinton, S. M. (2014). Farming for Ecosystem Services: An Ecological Approach to Production Agriculture. *BioScience*, *64*(5), 404–415. https://doi.org/10.1093/biosci/biu037

Pimentel, D., & Burgess, M. (2013). Soil Erosion Threatens Food Production. *Agriculture*, *3*(3), 443–463. https://doi.org/10.3390/agriculture3030443

Razumova, O. V., Alexandrov, O. S., Divashuk, M. G., Sukhorada, T. I., & Karlov, G. I. (2016). Molecular cytogenetic analysis of monoecious hemp (Cannabis sativa L.) cultivars reveals its karyotype variations and sex chromosomes constitution. *Protoplasma*, *253*(3), 895–901. https://doi.org/10.1007/s00709-015-0851-0

Riemens, M. M., Dueck, T., & Kempenaar, C. (2008). Predicting sublethal effects of herbicides on terrestrial non-crop plant species in the field from greenhouse data. *Environmental Pollution*, *155*(1), 141–149. https://doi.org/10.1016/j.envpol.2007.10.034

Riemens, M., Sønderskov, M., Moonen, A.-C., Storkey, J., & Kudsk, P. (2022). An Integrated Weed Management framework: A pan-European perspective. *European Journal of Agronomy*, *133*, 126443. https://doi.org/10.1016/j.eja.2021.126443

Rife, T. W., & Poland, J. A. (2014). Field Book: An Open-Source Application for Field Data Collection on Android. *Crop Science*, *54*(4), 1624–1627. https://doi.org/10.2135/cropsci2013.08.0579

Schutter, O. D. (2010). (rep.). *Human Rights Council*. United Nations.

Silva, Erin M., and Virginia M. Moore. “Cover crops as an agroecological practice on organic vegetable farms in Wisconsin, USA.” *Sustainability* 9.1 (2017):55.

Smith, P., & Gregory, P. J. (2013). Climate change and sustainable food production. *Proceedings of the Nutrition Society*, *72*(1), 21–28. https://doi.org/10.1017/S0029665112002832

Storkey, J., Meyer, S., Still, K. S., & Leuschner, C. (2012). The impact of agricultural intensification and land-use change on the European arable flora. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1732), 1421–1429. https://doi.org/10.1098/rspb.2011.1686

Stratton, A. E., Comin, J. J., Siddique, I., Zak, D. R., Dambroz Filipini, L., Rodrigues Lucas, R., & Blesh, J. (2022). Assessing cover crop and intercrop performance along a farm management gradient. *Agriculture, Ecosystems & Environment*, *332*, 107925. https://doi.org/10.1016/j.agee.2022.107925

USDA. (2022). (rep.). *National Hemp Report*. USDA.

Van Duivenbooden, N., Pala, M., Studer, C., Bielders, C. L., & Beukes, D. J. (2000). Cropping systems and crop complementarity in dryland agriculture to increase soil water use efficiency: a review. *NJAS: Wageningen Journal of Life Sciences*, *48*(3), 213–236.

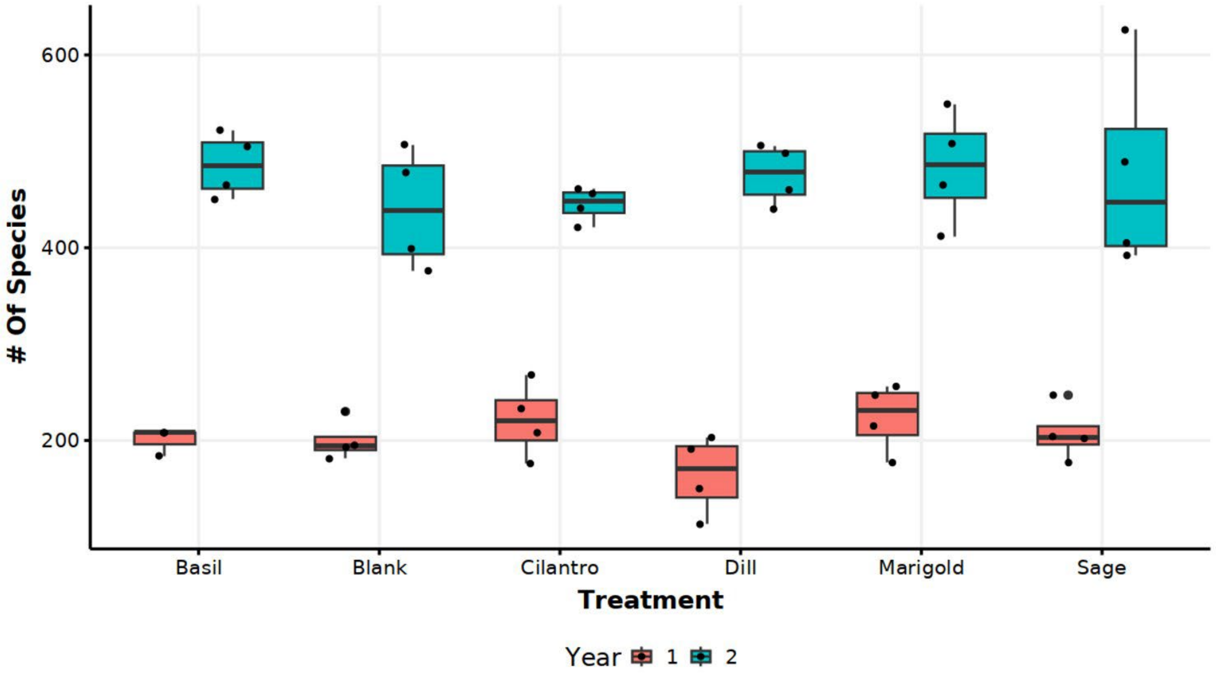
Verret, V., Gardarin, A., Pelzer, E., Médiène, S., Makowski, D., & Valantin-Morison, M. (2017). Can legume companion plants control weeds without decreasing crop yield? A meta-analysis. *Field Crops Research*, *204*, 158–168. https://doi.org/10.1016/j.fcr.2017.01.010

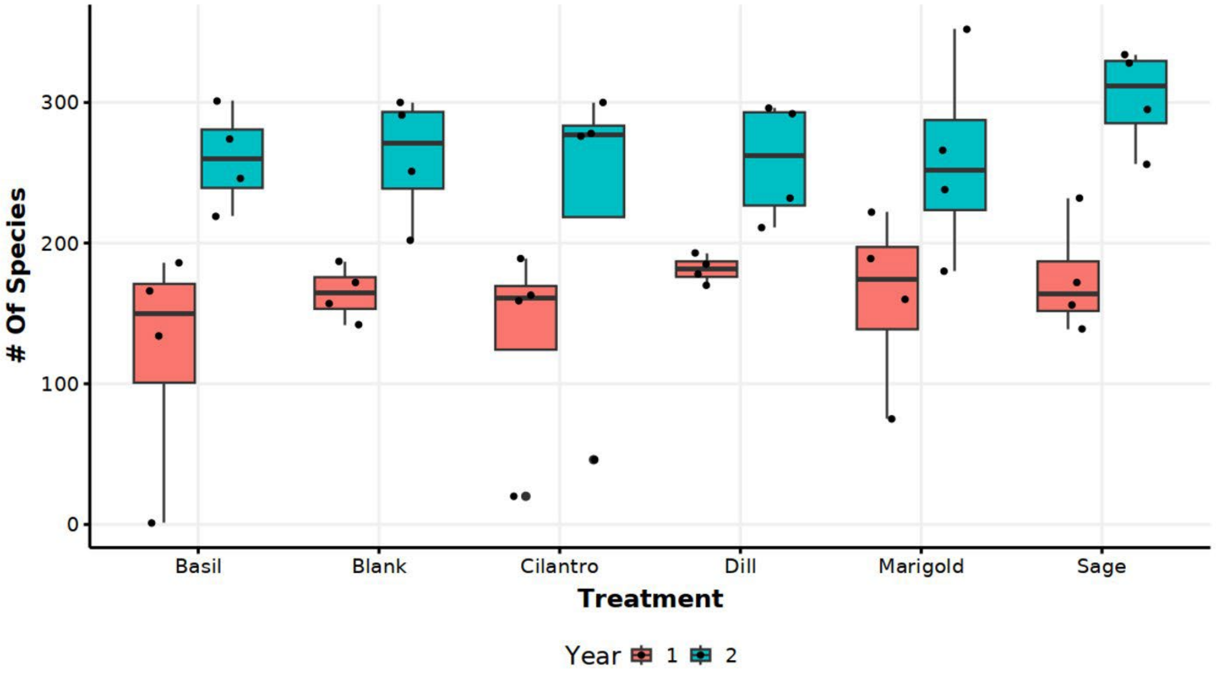
Wickham, H. (2011). *Ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics*. Wiley Interdisciplinary Reviews: Computational Statistics. https://cran.r-project.org/web/packages/ggplot2/ggplot2.pdf

Woolley, J., & Davis, J. H. C. (1991). The agronomy of intercropping with beans. *Common beans: research for crop improvement.*, 707-735.

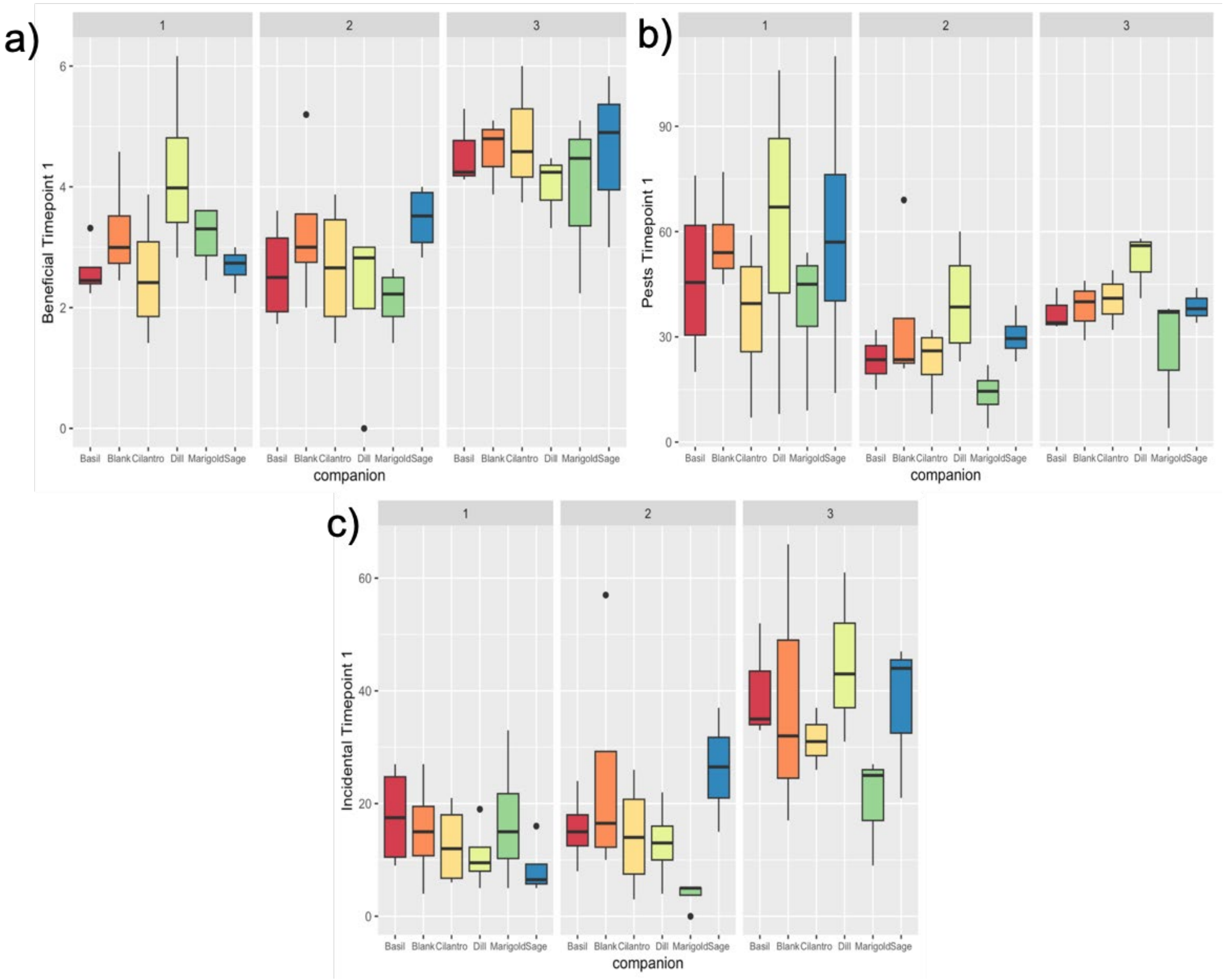
Yeganehpoor, F., Salmasi, S. Z., Abedi, G., Samadiyan, F., & Beyginiya, V. (2015). Effects of cover crops and weed management on corn yield. *Journal of the Saudi Society of Agricultural Sciences*, *14*(2), 178–181. https://doi.org/10.1016/j.jssas.2014.02.001

Appendix A





Supplementary Figure 1: (a) 16S and (b) ITS number of species between years.



Supplementary Figure 2: Boxplots of all three environments for each insect category for timepoint 1: (a) beneficial, (b) pest, (c) incidental

Supplementary Table 1: Raw data used for analysis.

location	Year	Environment	companion	block	companion_harvest	Companion_Price
WMARS	2022	1	Basil	1	5.62	359.68
WMARS	2022	1	Basil	2	5.4	345.6
WMARS	2022	1	Basil	3	5.38	344.32
WMARS	2022	1	Basil	4	5.19	332.16
WMARS	2022	1	Blank	1	0	0
WMARS	2022	1	Blank	2	0	0
WMARS	2022	1	Blank	3	0	0
WMARS	2022	1	Blank	4	0	0
WMARS	2022	1	Cilantro	1	0.91	43.68
WMARS	2022	1	Cilantro	2	0.73	35.04
WMARS	2022	1	Cilantro	3	2.96	142.08
WMARS	2022	1	Cilantro	4	1.06	50.88
WMARS	2022	1	Dill	1	2.01	96.48
WMARS	2022	1	Dill	2	1.93	92.64
WMARS	2022	1	Dill	3	1.78	85.44
WMARS	2022	1	Dill	4	0.84	40.32
WMARS	2022	1	Marigold	1	25.5	77.5
WMARS	2022	1	Marigold	2	34.5	105
WMARS	2022	1	Marigold	3	25.5	77.5
WMARS	2022	1	Marigold	4	28.5	87
WMARS	2022	1	Sage	1	0.66	31.68
WMARS	2022	1	Sage	2	0.88	42.24
WMARS	2022	1	Sage	3	0.47	22.56
WMARS	2022	1	Sage	4	0.54	25.92
WMARS	2021	2	Basil	1	11.14	712.96
WMARS	2021	2	Basil	2	10.17	650.88
WMARS	2021	2	Basil	3	8.95	572.8
WMARS	2021	2	Basil	4	12.47	798.08
WMARS	2021	2	Blank	1	0	0
WMARS	2021	2	Blank	2	0	0
WMARS	2021	2	Blank	3	0	0
WMARS	2021	2	Blank	4	0	0
WMARS	2021	2	Cilantro	1	3.67	176.16
WMARS	2021	2	Cilantro	2	3.87	185.76
WMARS	2021	2	Cilantro	3	3.04	145.92
WMARS	2021	2	Cilantro	4	3.95	189.6
WMARS	2021	2	Dill	1	4.51	216.48
WMARS	2021	2	Dill	2	5.71	274.08

WMARS	2021	2	Dill	3	4.37	209.76
WMARS	2021	2	Dill	4	1.65	79.2
WMARS	2021	2	Marigold	1	23.5	71.5
WMARS	2021	2	Marigold	2	14.4	44
WMARS	2021	2	Marigold	3	20.8	63.5
WMARS	2021	2	Marigold	4	24.5	74.5
WMARS	2021	2	Sage	1	1.53	73.44
WMARS	2021	2	Sage	2	1.04	49.92
WMARS	2021	2	Sage	3	0.86	41.28
WMARS	2021	2	Sage	4	0.82	39.36
LCO	2021	3	Basil	1	3.925	251.2
LCO	2021	3	Basil	3	4.26	272.64
LCO	2021	3	Basil	4	5.025	321.6
LCO	2021	3	Blank	1	0	0
LCO	2021	3	Blank	3	0	0
LCO	2021	3	Blank	4	0	0
LCO	2021	3	Cilantro	1	2	96
LCO	2021	3	Cilantro	3	2.9	139.2
LCO	2021	3	Cilantro	4	2.1	100.8
LCO	2021	3	Dill	1	1.1	52.8
LCO	2021	3	Dill	3	1.4	67.2
LCO	2021	3	Dill	4	3.13	150.24
LCO	2021	3	Marigold	1	15.6	47.5
LCO	2021	3	Marigold	3	33	100.5
LCO	2021	3	Marigold	4	24.3	74
LCO	2021	3	Sage	1	0.46	22.08
LCO	2021	3	Sage	3	0.555	26.64
LCO	2021	3	Sage	4	1.195	57.36

Location	weed_cover	plant_height	dry_weight	bucked_weight	Total_CBD
WMARS	36.67	118.4	1.525	0.9	5.63409
WMARS	33.33	135	1.675	1.1	7.17203
WMARS	40	129.4	2.55	1.3	8.48310
WMARS	38.33	98.2	1.7	0.8	8.85501
WMARS	86.67	146.2	2.075	1.2	9.31559
WMARS	38.33	114.4	1.475	0.6	8.86000
WMARS	93.33	102.4	2.2	1.05	7.76095
WMARS	93.33	94	0.9	1	6.64774
WMARS	60	120	1.45	0.85	7.26633
WMARS	51.67	129.6	2.25	0.7	6.14157
WMARS	48.33	138	2.05	0.8	6.44964
WMARS	76.67	97.4	1.75	0.85	6.86338
WMARS	48.3	155	1.85	0.6	6.60719
WMARS	36.67	163.8	1.45	0.7	6.44727
WMARS	35	119	2.575	1.35	8.33710
WMARS	51.67	92.6	1.725	1.15	10.79110
WMARS	36.67	144.4	2.45	1	8.20298
WMARS	18.33	136.6	2.125	0.65	6.39480
WMARS	38.33	108.8	1.05	0.5	10.62877
WMARS	25	87.2	1.75	0.65	2.90846
WMARS	56.67	155.6	2.225	1.05	6.61726
WMARS	40	134.6	2.1	1.15	5.20639
WMARS	85	127	1.6	0.8	11.23781
WMARS	31.67	168.2	1.03	0.335	8.186415

WMARS	**63.33**	**89**	**0.9**	**0.55**	**5.60139**
WMARS	6.67	160.4	1.31	0.545	7.49294
WMARS	18.3	162.6	0.65	0.27	8.547325
WMARS	0	160.8	1.08	0.395	7.82425
WMARS	18.3	132.2	0.43	0.125	9.191335
WMARS	15	151.4	0.88	0.25	8.76227
WMARS	61.67	175.8	0.72	0.26	9.10802
WMARS	48.3	144.2	1.10	0.42	11.69588
WMARS	15	152.6	0.75	0.335	8.872565
WMARS	26.67	162.2	0.79	0.31	9.291335
WMARS	6.67	147.2	1.02	0.31	11.478955
WMARS	95	149.4	0.75	0.275	9.612805
WMARS	55	129.6	0.02	0.015	9.23441
WMARS	25	150.6	0.82	0.34	4.902165
WMARS	8.3	161	0.87	0.34	6.35909
WMARS	30	166.6	0.74	0.235	6.876175
WMARS	18.3	135.8	0.92	0.36	6.342245
WMARS	0	153.2	0.69	0.185	11.54874
WMARS	3.3	156.8	1.71	0.705	10.328795
WMARS	5	141.8	0.36	0.115	8.66302
WMARS	6.67	146.2	0.45	0.16	4.483475
WMARS	8.3	175.8	0.74	0.3	8.907325
WMARS	23.3	155.6	1.80	0.72	6.99802
WMARS	50	141.6	0.46	0.145	10.047645

LCO	**11.67**	**136.2**	**1.43**	**0.661**	**10.548635**
LCO	10	122.8	0.94	0.606	11.22695
LCO	56.67	103	1.38	0.772	11.51917
LCO	33.33	121	1.38	0.717	14.5181
LCO	40	132.4	2.31	1.213	10.899705
LCO	20	101.2	1.43	0.717	11.871015
LCO	31.67	125.2	1.32	0.551	13.682405
LCO	38.33	135.6	1.16	0.606	13.653635
LCO	18.33	134.8	1.98	0.992	12.89187
LCO	70	155.2	2.20	0.882	14.762325
LCO	16.67	130.4	1.54	0.717	14.229945
LCO	26.67	133	1.76	0.827	10.37917
LCO	5	147.6	1.71	0.717	11.391095
LCO	8.33	106.4	1.32	0.606	13.966255
LCO	5	130.6	1.27	0.661	13.697325
LCO	38.33	138.25	2.09	1.0472	9.362245
LCO	85	114.4	1.05	0.606	12.89187
LCO	13.33	139.6	1.32	0.551	11.51917

location	Beneficial_1	Pest_1	Incidental_1	Beneficial_2	Pest_2	Incidental_2
WMARS	11	20	11	10	20	6
WMARS	6	76	24	4	14	1
WMARS	5	57	27	12	49	11
WMARS	6	34	9	2	43	6
WMARS	6	77	13	30	19	18
WMARS	10	45	4	28	47	10
WMARS	8	57	27	7	30	9
WMARS	21	51	17	27	49	5
WMARS	8	7	6	10	10	3
WMARS	15	59	7	17	36	11
WMARS	4	32	21	11	57	5
WMARS	2	47	17	16	19	1
WMARS	13	54	19	27	37	8
WMARS	19	106	9	18	30	44
WMARS	8	80	5	11	49	1
WMARS	38	8	10	19	57	4
WMARS	13	41	33	24	10	11
WMARS	6	9	5	13	13	6
WMARS	13	49	18	7	10	3
WMARS	9	54	12	13	8	0

WMARS	8	14	6	27	5	21
WMARS	9	65	7	12	22	4
WMARS	7	110	16	22	36	2
WMARS	5	49	5	7	113	3
WMARS	13	21	16	34	12	8
WMARS	4	15	8	8	10	3
WMARS	3	32	24	8	13	3
WMARS	9	26	14	18	12	7
WMARS	9	24	20	69	3	2
WMARS	4	21	10	9	5	3
WMARS	27	69	57	21	5	2
WMARS	9	23	13	3	3	8
WMARS	4	8	3	32	3	6
WMARS	2	32	9	83	4	4
WMARS	15	23	26	51	5	4
WMARS	11	29	19	21	6	17
WMARS	9	30	12	20	3	4
WMARS	0	23	4	7	5	8
WMARS	9	60	22	13	8	3
WMARS	7	47	14	11	2	6
WMARS	4	4	5	11	8	3
WMARS	2	16	0	27	3	5
WMARS	7	13	5	1	8	4
WMARS	6	22	5	23	14	0
WMARS	10	23	30	10	4	1
WMARS	16	28	23	24	1	2
WMARS	8	31	15	15	4	3
WMARS	15	39	37	23	20	5
LCO	17	33	35	5	6	3
LCO	18	44	33	3	16	4
LCO	28	34	52	2	4	1
LCO	23	40	32	5	2	1
LCO	15	29	17	16	1	1
LCO	26	46	66	11	5	1
LCO	36	41	26	12	5	2
LCO	14	49	31	13	3	6
LCO	21	32	37	4	4	1
LCO	20	58	61	11	1	4
LCO	11	41	31	25	3	7

LCO	18	56	43	8	1	0
LCO	26	38	25	4	0	3
LCO	5	4	9	6	0	1
LCO	20	37	27	4	1	1
LCO	34	38	44	9	1	2
LCO	9	34	21	8	4	4
LCO	24	44	47	8	4	1