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SIMPLE ORGANIC FERTILIZER AMENDMENTS FOR FARMING IN DEGRADED SOILS: EFFECTS ON PLANT-MICROBE INTERACTIONS

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By

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SIMPLE ORGANIC FERTILIZER AMENDMENTS FOR FARMING IN DEGRADED SOILS: EFFECTS ON PLANT-MICROBE INTERACTIONS

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ABSTRACT

SIMPLE ORGANIC FERTILIZER AMENDMENTS FOR FARMING IN DEGRADED SOILS: EFFECTS ON PLANT-MICROBE INTERACTIONS

By

Andrew J. Adamski

The rapid loss of topsoil, biodiversity, and water quality across agricultural land in the United States and the industrialized world poses some of the most important risks to the future of global and domestic food security. Not only is arable land being lost at an unsustainable pace, but the average age of farmers has also steadily been rising due to a myriad of barriers young, BIPOC (Black, Indigenous, and People of Color), and beginning farmers face. In an attempt to address these issues, worm castings, bokashi tea, fish hydrolysate, and biochar were applied alone and in combination to an extremely degraded soil system at Northern Michigan University's Agricultural Field Plot. These amendments can all be made with very limited monetary input while providing a significant amount of nutrients, and microbial diversity to the soil. Overall plant biomass, culturable microbial diversity, microbial functional diversity via community-level physiological profiling, soil water holding capacity, soil microbial carbon, microbial biomass carbon, and soil carbon dioxide fluxes were all hypothesized to respond significantly to applications of any of the treatments. The use of worm castings as a whole resulted in the most significant effects on plant performance, total microbial diversity, and soil water-holding capacity while worm castings in conjunction with biochar had even more significant positive effects on plant growth and soil water-holding capacity than any other treatment. These results suggest that these organic soil amendments can increase soil and plant health which provides a cost-effective strategy for both pre-existing and newly established agricultural areas.

Keywords: Microbial Ecology, Plant-Microbe Interactions, Microbial Diversity, Urban Farming

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This thesis follows the formatting required by the *Nature* Journal

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Introduction:

Agricultural production across the world has a long history of overuse of inorganic fertilizers and pesticides[1]. These practices have reduced the quality of the soil which have led to the Midwestern United States alone losing 57.6 billion metric tons of soil in a mere 160 years at an annual rate of 5 tons/acre/year[2]. Many of these issues are a result of commonly used industrialized agricultural practices. These models force a reliance on synthetic fertilizers and the extermination of unwanted plants to grow a single crop, such as corn and soybeans. Moreover, farmers the world over are seeing their land and livelihoods disappear due to climate change, suburban sprawl, topsoil loss, record droughts, and rapid desertification[3]. The culmination of these factors leads to a need for alternative forms of agriculture centered around diverse crop mixes, annual and perennial cover crop practices, soil restoration efforts, and fair market values. This project is a small step in addressing some of these issues by examining the influence of organic soil amendments using appropriate technology, traditional ecological knowledge, and diverse, polycultural growing methods in otherwise non-arable soil.

Urban communities that do not have resilient, diversified farms nearby result in a dependence on fast food and convenience stores for food that only supply empty calories without the necessary vitamins and minerals that fresh food provides[4]. Even urban communities with ready access to grocery stores experience higher prices for quality local produce[5]. By growing the food in as close proximity to a population center as possible, some of these issues may be reduced. However, there are hurdles that many urban farmers face when trying to startup. Some of the most contaminated soils in the world are found in urban and suburban areas where previous industrial oversight has resulted in legacy chemicals and heavy metal deposits being commonplace in now vacant urban areas[6]. Additionally, urban farmers often are faced with

extremely low nutrient baselines in their soils due to years to backfill and lack of cultivation in the first place. Therefore, the location of this study was placed near a city center with a long history of nutrient depletion where demonstrable improvements to soil quality and crop yield would be most reflective of the conditions urban farmers are most likely to encounter.

In 2008 the United Nations identified food security as a major risk to global security when they established the High-Level Task Force on Global Food and Nutrition Security (HLTF). This task force identified food security in developing nations as a major risk due to developed countries' subsidy programs and trade tariffs which predispose world food markets to price spikes and lack of food availability. They also identified climate change-related effects, such as drought, flood, desertification, and environmental degradation as being a greater risk to poorer countries and areas with higher population densities[7]. Almost all of these factors describe the American ecosystem. Our food supply is unstable at best, our soils are degrading at unprecedented rates, and 80% of our population lives in areas that are deemed highly susceptible to climate change disaster by this same UN report. Climate change could cause major food shortages all across the world due to the inability of the common commodity food crop cultivars to adapt to highly variable weather patterns[8]. A plethora of studies have shown that conventional cropping systems underperform organic systems in weather patterns that deviate at all from the mean[9–13]. Additionally, trade wars, and ground wars, between major food-producing countries sparks unhindered production of commodity crops for the global marketplace, bottoming out the prices due to overproduction or causing price spikes due to blockades[14]. This practice significantly decreases the production of food meant to be marketed through local communities due to more farmers growing commodity crops for resale in external markets[15] If we truly wish to establish an agricultural paradigm that can feed the world, we need to start with the soil.

One outcome of common industrialized agricultural practices is the rapid loss of topsoil and organic matter in agricultural soils[16]. Soil organic matter is the top fraction of the soil which contains the rhizosphere or root zone. It is here that archaea, bacteria, fungi, protozoa, plants, and animals coexist and create the infinitely complex nutrient and enzyme webs inherent to healthy soils[17,18]. Farming practices that prioritize soil health and biological diversity consistently produce healthier[19], more nutritious crops[20], more efficiently and at a lower cost than large-scale commodity cropping operations[21,22].

Organic farming emphasizes soil health as the foundation of quality food production. Farmers who adopt organic practices implement various strategies to ensure that the organic matter in their soils remains at high levels[23]. Most of these strategies share adding organic matter and microbial diversity to the soil as the main driving factor. Whole ecosystem diversity is also an important tenet of organic farming. Organic farmers utilize cover crops and diverse crop rotations as a means to control weeds and provide habitat for beneficial insects and soil flora[24]. These cover cropping systems are intended to mimic natural ecosystems in terms of diversity and ecosystem services through trophic cascade dynamics and functional variations in nutrient cycling, pest and disease redirection, beneficial insect and microbe attraction, synergistic rhizosphere couplings, and complex nutrient cycling through metabolic cascade dynamics. In addition to cover cropping as a way to maintain existing nutrient levels and in some cases enhance them, farmers also utilize fertilizers to add nutrients back to the soil that cover crops take out. The fertilizers that farmers apply to their fields have the most direct influence on the diversity and abundance of soil flora[25,26]. The main difference between organic and conventional fertilizers lies in the nutrient complexities. Organic fertilizers mimic natural root exudates and microbial secretions, while conventional fertilizers are pared down to salt-based water-soluble

renditions of macro and micronutrients found in the ecosystem. Most organic farmers use composted animal manure as the major fertilizer, while some are starting to add mineralized rock dust, biochar, liquid compost teas, and other amendments[27]. These animal composts are rich in beneficial bacteria, archaea, fungi, and nematodes as well as soluble and organically bound essential nutrients[28] .

Typical fertilizers in commodity agriculture and in urban and suburban lawnscapes, such as ammonia, potash, and monocalcium phosphate, are concentrated forms of nutrients essential to basic biological functions. Since 1960, the total amount of phosphorus application has doubled and total nitrogen, and potassium applications have tripled in agricultural fields. Native soil microbes often cannot handle the acidification and hypertonic solution that these fertilizers create[29]. This results in a drastic reduction of soil microbial taxonomic and functional diversity, abundance of total microbes, and organic matter[26]. These are important factors in any soil ecosystem, especially agricultural soils, because microbes provide resilience against pathogens[30], break down organic matter[31], and are key players in every major nutrient cycle[32,33]. As a result, modern agricultural practices are becoming more and more dependent on synthetic fertilizers and pesticides to provide the fertility that native soil microbes would otherwise make accessible.

Low levels of organic matter in urban and agricultural soils present major challenges for farmers now and will continue into the future[34]. This issue is especially common in urban agricultural soils because of long-term disturbance and structural instability[35]. Many of the common land-use practices in urban soils mirror agricultural soils. Overapplication of salt-based fertilizers, heavy use of synthetic herbicides, degradation of organic matter, and monocultural replications of natural ecosystems persist. Most urban farms attempt to start in areas that are either contaminated waste sites or were managed as fertilizer and pesticide-dependent lawns

before being abandoned, with back-filled residential plots being commonplace as well. Major advances have been made in remediating contaminated soils by continuous applications of bacteria and fungi selected to break down a specific chemical contaminant[36–38]. However, these treatments often take years to work, and crops for human consumption cannot be harvested from these sites until all traces of the contaminant have been eliminated. The ideal urban farm is established in an area where there are no hazardous contaminants. However, many urban soils have been backfilled after a building demolition or have experienced years or even decades of synthetic inorganic fertilizer and pesticide applications leaving the soil devoid of native microbial populations. This sterilization event necessitates a significant financial and personal investment by the aspirant farmer. Soils subjected to these stresses lack any significant amounts of organic matter and microbial diversity[39] rendering their basal capabilities lacking in a stable foundation for success. It takes a year or more for urban farmers to build their soils[40]. This is often too long and results in the failure of many upstart farms unless major capital investments are made upfront to build the soil. With such small profit margins in farming, it is often not feasible to make large capital investments upfront. Worm castings, biochar, fish hydrolysate, and bokashi tea are all organic fertilizers that can provide organic matter and microbial diversity necessary for an agroecosystem to thrive without major initial investments can be readily made in small areas, and can use waste products from other local businesses[41–43]. Combinations of these amendments are the most effective and can even result in significant yields of crops, even those with the heaviest nutrient requirements, within the first year of application.

Explanation of Treatments

Worm castings are usually a waste product of red wiggler worms (*Eisenia fetida*). Other worm species are used, but not as often as *E. fetida.* These worms are efficient digesters of organic waste material; a resource commonly underutilized in vegetable production. The nutrient dense and microbially diverse feces of these worms are collected and used as a fertilizer in some organic farming systems[44]. This method is preferred by urban farmers because compost produced from mammal feces requires a large area, large machinery, and, if not done correctly, can be a major source for contamination by pathogens. Worms digest the organic matter which results in a 500-fold increase in bacterial biomass[45]. This process significantly reduces the time to completion compared to standard hot composting methods, as well as provides an easy way for garden and yard waste to be recycled. Worms have long been at the center of soil structure and function. Being detritivores, these organisms have evolved complex biochemical pathways to extract nutrients from dead and decaying plant material that otherwise may not be bioavailable[46]. Earthworms also disperse nutrients throughout a soil due to their much larger bodies than other decomposers. The tunneling effects they have also play a significant role in aeration of soil. Earthworms also distribute microbial diversity throughout soils[47]. This effect leads to colonization of microbes into depleted soils which can help stimulate nutrient cycling, plant growth factors, and trophic cascades[44]. Each of these effects leads to increased diversity, health, and functionality of soils that worms are present in. Earthworm castings can be useful in introducing microbial diversity to a soil but lack the other benefits of having worms themselves present in the soil.

Worm castings were decided on as the choice for organic soil amendment in this experiment because of the ease of production, consistency of product, and availability in urban

farming environments. The functional aspect was to provide a stable, organic substrate for microbial populations to proliferate and harbor complex plant-microbe interactions that are often suppressed in heavily mineralized soils such as found in the parent material in the experiment site. The existing microbial populations in the worm castings and the readily available suite of complex organic compounds were hypothesized to create a stable starting point for soil remediation and nutrient availability. Additionally, the availability of nutrients and microbes in the worm casting material was hypothesized to benefit when applied in combination with the other soil amendments listed below, either by increasing the diversity of microbial population and therefore nutrient cycling capabilities and/or by increasing the complex organic material substrate required to harbor complex soil microbial communities.

Biochar, a stabilized carbon matrix usually produced from pyrolysis of cellulosic plant material, has been used for centuries and across many cultures as an inexpensive and easy way to introduce complex carbon molecules into a depleted agricultural soil. In the Amazon basin, samples of subsoil amended with biochar, known as "Terra Preta", have been mixed with composted human and animal feces, as a primary fertilizer in their farming practices[48]. These people have been hypothesized to be the ancestors of the Maya and Inca cultures, both of which were able to support massive population densities for their time without industrialized agriculture and centralized septic systems[49]. Utilizing biochar was hypothesized to establish and introduce a matrix of carbon-based complexes in the rhizosphere. Introduction of biochar would therefore solidify a stable environment for microbial populations to establish and proliferate when inoculated into the system. However, because of the intensive catabolic process biochar must go through during production, it does not introduce any microbial populations of its own[50]. This lack of inherent microbes in the biochar requires an external inoculation event often referred to as

"activating" or "activated biochar"[51]. Most often this is achieved by mixing the raw biochar product with fully mature compost or soaking with a compost tea preparation. For the sake of this experiment, the biochar was not activated before introduction to the soil and the worm casting compost was used in WCBC in conjunction with the biochar to simulate the 'activated biochar' product.

Fish hydrolysate is a form of fish byproduct fertilizer adapted from traditional fish-based preparations used by indigenous peoples across the world for millennia to fertilize their crops[52]. As is the case when it comes to utilizing any protein-rich and microbial diverse substrate for agricultural purposes, a sanitation step is required to ensure contamination by pathogens and unwanted bacteria does not occur. Modern techniques employ enzymatic reactions catalyzed by specific temperatures, oxygen levels, pressure, and bacterial or enzyme presence[53]. Depending on the specifics of each variable and starting substrate used, the resulting hydrolyzed product can be vastly different. Because of this, many fish hydrolysate products currently exist on the market, each with slightly different nutrient profiles and modes of action[54]. From an industrial perspective, this allows for many products to be offered without copyright and product originality claims being commonplace. The downside being that no two products are exactly the same and even a slight change in the process can result in a different product, leading to uncertainty among farmers and therefore hydrolysate products are rarely used in modern agriculture[55].

Commercial fish hydrolysate was used for this experiment, but anyone can make their own fish hydrolysate fertilizer. The process only requires discarded fish heads and guts, an old blender, non-chlorinated well water, a simple carbohydrate (molasses is commonly used as it is unprocessed and unbleached), and a sanitized plastic bucket with a sealable lid[56]. The fish

byproduct is blended to a liquid consistency, then mixed with water in a bucket at, ideally, a 1:5 fish to water ratio, then molasses is added at a 1:30 ratio to the fish-water mix. The product is stirred together, then the lid placed securely over the top. The entire process can take anywhere from 3 to 10 weeks depending on the ambient temperature. Given the abundance of unused fish waste from the fishing industry, just about anyone living within 50 miles of a water source should be able to produce this high-quality fertilizer.

Application of fish hydrolysate can be done at almost any time of the plant life cycle. Many vegetable farmers will dilute with water and use in the seedling nursery to provide valuable macronutrients to the quickly growing plants[57]. Others will mix the hydrolysate in a solution when transplanting in the field as an alternative to pelletized salt fertilizer. This serves a major function in plant growth: the plant's "transplant shock" response is lessened[58–60], allowing for vegetative and root growth up to 14 days earlier than when watered in with just water and no additional nutrient source. This is in opposition to most salt based fertilizers that can cause a shock to the plant's system during vegetative growth phases[61,62]. Due to the abundance of soluble nitrogen and potassium, the plant will sometimes have to increase water uptake to offset the hypertonic solution created in the cytoplasm, which not only increases the water requirements of the plant, but also causes a deficiency in other nutrients such as calcium and magnesium[63,64]. The resulting plant is therefore susceptible to fungal and insect pest pressure[65]. The N, P, and K found in fish hydrolysate are not found in readily soluble salt forms and therefore the release and uptake by the plants via their transmembrane adsorption of macromolecules allows for a slower and more sustained rate of incorporation into the plant tissue[66–68]. Not only does this allow for a more resilient growth pattern, but also increases the stability and duration of the macro and micro nutrients found in the hydrolysate material. This stability is further enhanced when presented in a

soil with high organic matter content and microbial biomass, as the native microbes in the soil are able to cycle those nutrients through multiple nutrient cycling pathways when not immediately needed by the plant[69,70]. The last common method for fish hydrolysate application is via foliar spray application. This solution is commonly applied at a dilution rate of 1:10 parts fish hydrolysate to water. Because the more complex N, P, and K macronutrients are bound in larger protein and lipid structures, the plant is able to absorb these molecules through the stomata or through the cuticle of the leaf. When amendments are applied this way, the plant is able to distribute and utilize the nutrients at a rapid rate and the risk of leaching through the soil or loss due to over application is diminished.

Bokashi tea is a broad term for a group of fermented preparations commonly used in Korean Natural Farming (KNF), and Japanese Organic Farming systems[71]. These preparations date back centuries and have been constantly refined through experimentation and natural observations. The basic premise of the bokashi preparation is to take food waste products and ferment them in a way that produces a highly concentrated inoculant to bolster living soils, compost piles, and growing plants via foliar feeding or rootzone drips[72]. Bokashi, in its most basic form, involves inoculating the desired organic matter material with a selection of Lactobacilli bacteria[73]. These bacteria thrive in anaerobic environments and are widely regarded for their ability to produce complex fermentation byproducts (i.e. yogurt, kefir, kombucha, kimchi, etc.)[74]. The containment of the organic matter in an anaerobic fermentation vessel allows for a faster process and more complete retention of macro and micro nutrients in the starting substrate[75]. The flexibility of the process also allows for multiple stages of the process to be carried out simultaneously at different rates, meaning the practitioner can have a constant supply of bokashi amendments for the desired application at different times of the year. Another

significant benefit of the bokashi method for home composters and industrial composters alike is its ability to utilize a wider array of food scrap substrates[76]. This is achieved through a homolactic fermentation, which is a more complete and hostile environment for pathogens such as *E. coli* and *Salmonella spp.,* the most common contaminating bacteria in home composts when meat and other animal products are added, outside of the recommendations. Additionally, citrus rinds and other oily substances are generally not recommended for aerobic composting as the oils can harm the beneficial bacteria and the oils can create pockets of anaerobic fermentation, which when uncontrolled is more susceptible to unwanted and potentially hazardous byproducts and bacteria[77].

Grow Juice 2© is a proprietary blend of macro and micro nutrients produced via fermentation hydrolyzation similar to bokashi and fish hydrolysate production methods. The intent of including it in the experiment was to compare the previous homemade (or easily homemade) preparations with an experimental industry product.

Hypotheses:

I hypothesized that the addition of any amendments would increase plant yield, increase microbial taxonomic and functional diversity, soil organic matter carbon, microbial biomass, soil water holding capacity, and soil CO_2 flux.

METHODS

Experimental Design

Nine total treatments were applied: control (C), worm castings alone (WC), worm castings and bokashi (WCBO), worm castings and fish hydrolysate (WCFH), worm castings and biochar (WCBC), bokashi alone (BO), fishy hydrolysate alone (FH), biochar alone (BC), and Grow Juice ® (GJ). There were 10 replicates of each of the 9 different treatments for a total of 90 plots (Table 1).

Site Preparation

Soil at Northern Michigan University's Agricultural Field Plot was prepared by digging 1 meter deep and inverting O, A, and B horizons. The O and A horizon were minimal in these soils due to extended, heavy use of the land as a landfill and a previous backfill event with coarse grain sand. Any metal, rubber, plastic, or other non-organic debris were removed. The mixed soil was then put back into each hole after a lining of perforated contractor grade plastic bags were inserted to minimize leaching of treatments and extraneous inputs. Bags were perforated at the bottom to allow for water drainage to avoid anaerobic soil conditions. Nine treatments with ten replications of each were then applied to each soil system as shown in Table 1. Soils were then watered in and allowed to rest for 1 month before planting. Black oil sunflowers, Cherokee trail of tears pole beans, and black beauty zucchini summer squash (all from Seed Savers Exchange Inc. Decorah, Iowa) were planted June 20th 2016 after the last threat of frost had passed. Seeds were watered in and re-watered every three days or as needed throughout the rest of the summer. Any weeds were pulled as soon as they were noticed. Due to moderate deer and goose grazing events, dried coyote urine was applied to the perimeter of the test plots and pouches were hung from strings around the test area.

Sources and Use of Amendments

Liquid fertilizers (bokashi tea, fish hydrolysate, worm casting tea, Grow Juice 2 ©) were applied once as a root soak at planting. One liter of each was applied at a 1:10 dilution rate for consistent concentrations. Each liquid treatment was applied twice more as root soaks throughout the rest of the season at 50-day intervals to their respective plots at the same dilution ratio and volume as originally applied. For treatments that had both worm casting tea and another liquid amendment, 0.5 L of each was used instead of the 1 L used for each treatment by itself.

The worm castings used were sourced from Carney's Crawlers LLC (Appleton, Wi). At the time, they used red wigglers but have since changed production methods and now use African night crawlers for their worm casting productions. Worm casting tea was made for the liquid application using 1 kg of worm castings and suspended in 10 L of dH_2O using a permeable cloth bag.

For the sake of this experiment, the biochar was not activated before introduction of the soil and the worm casting compost was used in WCBC in conjunction with the biochar to simulate the activated biochar product which was acquired through GreenQuest LLC in Richfield Wisconsin.

The fish hydrolysate used was DRAMM© 4-2-2 liquid fish fertilizer. This N-P-K ratio is the most common found between all of the different products on the market and DRAMM is headquartered in Two Rivers, Wisconsin so it was a logical source of a local, readily available product.

The bokashi preparation that we used was the most simple one described and consisted of uncooked rice soaked for 2 days in a dilution of raw cow's milk sourced from Full Circle Farm in

Seymour, Wi and water at a ratio of 1:3 parts milk to water. This process was used because the most basic premise of a bokashi preparation is to utilize Lactobacilli bacteria – which are abundant and diverse in raw cow's milk – and a carbohydrate source – usually wheat bran, rice, or molasses – as a substrate for the bacteria to ferment. We applied this mix at the same times as the fish hydrolysate and Grow Juice 2 during the growing season as a root drench to maintain consistency.

Harvest and Soil Collection

At the end of the growing season (27 September 2016) any plants that had grown were pulled while preserving as much root mass as possible by digging the whole plant using a trowel and then shaking the soil off of the roots. All plant organic matter was placed into a brown paper bag labeled with the plot number. Plants were dried in each bag at 37 ^{o}C for 48 hours. At the end of the drying period above ground biomass was measured by plant species from each plot as well as the below ground biomass. Average above ground biomass and below ground biomass were calculated by treatment type.

CO_2 Flux

Carbon dioxide fluxes were taken at 30-day intervals during the growing season starting 12 June 2016, the week before seeds were planted. A PP Systems EGM-5 portable CO_2 Gas Analyzer was used to collect CO_2 flux data[78]. Ambient CO_2 levels were measured before each collection period and every 15 minutes during collection. Gas collection collars were fabricated using 3.5" diameter PVC tubing cut to 8" long and a coupler to collect CO_2 flux was made using a PVC cap that fit firmly over the top of the 3.5" collars in the ground. A foam collar was glued to the inside of the coupler so that a consistent internal volume would be attained. The effluent tubes for gas exchange were inserted into holes drilled into the top and sealed using inert

silicone. Internal volume of the gas chamber was calculated by measuring the height above ground of each collar and adding the volume of the coupler.

Soil Water Percent

Soil water percent by weight was measured by taking 5 g of field moist soil, drying it, and measuring the difference in mass[79]. Field moist soil was determined to be moisture of the soil 12 hours after a soaking rain event. This occurred on 20 July 2016. Soil was taken from each plot using a trowel and 10 cm soil corer at 15 cm deep and transferred into plastic ziploc bags labeled by plot number. Soil was stored, sealed, at 4 ^{o}C for up to 1 week until tests were able to be run. Soil was sieved through a 5 mm screen to remove organic matter and small rocks. Five grams of the screened soil was then placed in aluminum boats made to be 4 cm in diameter. Samples were then placed in a thermo kiln at 105 ^{o}C for 12 hours. This caused any water to evaporate without oxidizing any organic matter or causing any material to off-gas resulting in loss of mass. The remaining dry soil was then weighed by pouring the sample into a tared weighboat. The difference in mass before and after drying was determined to be the total mass of water in the soil.

Microbial Functional Diversity

Analysis of soil microbial functional diversity was conducted using Biolog ® Ecoplates following the method described by Insam and Goberna (2004). Soil was collected from each plot on 3 September 2016 and stored in plastic ziplock bags labeled with the plot number. Samples were stored at 4 ^{o}C for up to 8 weeks. Five grams of soil were sifted through a 3 mm sieve and then funneled into sterile 50 ml centrifuge tubes. Tubes were labeled and stored at 4 ^{o}C until soil from all plots were in tubes. To separate bacteria from soil particles, 0.5 g of inert glass microbeads were added to each centrifuge tube along with 5 ml of 9% non-lactated Ringer's

Solution. Samples were shaken for 1 hour at 50 rpm and then centrifuged at 15 x *g* for 20 minutes. The remaining liquid was decanted off and stored in sterile test tubes labeled with the plot number at 4 ºC for up to one week. Once all 90 of the samples were ready, 120 µl of prepared solution was transferred to each well at 22 ºC. Thirty total Biolog® Ecoplates were used because each plate was inoculated with 3 different samples each as there were 3 sets of 31 different carbon substrates on each plate. Plates were measured at 590 nm on a Biotek Epoch II plate reader at 24, 48, 72, 96, and 120 incubation hour intervals. Optical Density (OD_i) measurements were corrected by subtracting the control, blank well, in each substrate group from each plate well. The average well color development (AWCD) of the solution absorbance value at 590 nm in each micro-plate well was used to to describe a proxy of the microbial functional diversity using the Shannon-Wiener Index (H), Evenness Index (E), and Richness index (S).

Culturable Microbial Diversity

A basic serial dilution method was done by sieving 1 g of each soil sample through 3mm sieve and then mixed in 10 ml dH_2O and then diluted again at 1:10, 1:100, and 1:1000 ratios[80]. Each dilution scale was then spread plated onto Potato Dextrose agar (PDA) using 1 ml of each of the three dilution scales onto a separate plate. Plates were then incubated at room temperature in a laminar flow hood to reduce contaminates for 48 hours. At which point total numbers of colony forming units, total counts of each unique colony type, and number of unique colony types on each plate were measured using a lab-made transilluminator.

Microbial Biomass Carbon

Soil microbial biomass carbon (SMBC) and soil organic matter carbon (SOMC) were measured using a modified version of the freeze-dried soil extraction method as written by Islam

et. al (1996). The same soil samples were used for this method as the Ecolog plate procedure. Five grams of oven-dried equivalent-weight sample of field-moist soil were placed in 20 ml round bottom boiling flasks and adjusted to 100% water holding capacity. The samples were frozen at -20 ^{o}C in the dark for 24 hours or until completely frozen. Samples were then freeze dried in a Labconco Freeze-Drier (model #75034) for up to 1 day or until no water was left in the sample. The freeze-dried and a separate 5.0 g oven-dried equivalent weight sample of field-moist soils were extracted with 20 ml 0.5 M K_2SO_4 (pH 7.0) in a 50 ml polycarbonate centrifuge tube by horizontal shaking (250 x g for 60 min). The soils suspensions were then centrifuged at 5000 x *g* for 10 minutes and then filtered to obtain soil-free extract. Organic C in these extracts was analyzed by a modification of the rapid oxidation spectrophotometric method of Heanes (1984) as follows: exactly 5.0 ml filtered extract was digested in a glass tube with 5 ml 0.17 M $K_2Cr_2O_7$ and 5 ml concentrated H_2SO_4. The glass tubes were heated in sand on a preheated hot plate at 135±5 ^{o}C for 30 min under the fumehood. A small glass funnel was kept in the mouth of each tube to help make sure the H_2SO_4 was able to be added consistently, drop by drop, to the tube containing the soil and dichromate mix. A modified burette held on a ring stand attached to another ring stand to act as a 3-axis arm was used to slowly add the H_2SO_4 to the dichromate-soil solution. After 5 ml of the sulfuric acid was added to the dichromate-acid solution the absorbance was measured at 590 nm. Sucrose solutions were also digested and used to standardize the absorbance readings of carbon content in the samples.

Statistical Analyses

One-way ANOVA analyses were done to compare the difference in means between treatment types for all response variables. The null hypothesis for each ANOVA test was that there was no significant difference in the means among treatments. Assumption of normality was

determined by visual observation of histograms and qq-plots of samples. A Tukey HSD test was done after each ANOVA test to identify differences in the mean among each of the different groups. The null hypothesis of each Tukey HSD test was that there was no significant difference in the mean between treatment types. The alpha for both the one-way ANOVA and Tukey tests was set to 0.05. Pearson correlation tests were done to compare relationships between each of the response variables. Multiple regression analysis was done using the variables mentioned above to explain variation in the data. All statistical analyses were conducted in IBM SPSS version 24 and R Studio version 4.2.2. R packages used can be found in the appendix.

Diversity Analyses

Shannon's diversity index proxy from the Biolog Ecoplates was calculated following the procedure as written by Insam and Goberna (2004). Plates from the 60 hour mark were used to calculate diversity and richness indices. Absorbance values at 590 nm were corrected for variable inoculum density by subtracting the well color development of the plates at 60 hours from the plates at 0 hours. ANOVA tests were done to compare species richness and diversity between well samples.

RESULTS

Plant Biomass

There was a significant difference in the mean of the plant biomass among treatment types ($F=5.919$, $p<0.001$, d.f.=8, 80) (Figure 1). WCBC resulted in the greatest biomass among all treatments while all treatments with worm castings were significantly greater than the control and treatments without worm castings. Treatments without worm castings did not differ from the control treatment.

Culturable Soil Microbial Diversity

There was a significant difference in the means of culturable microbial species richness between treatment types ($F=8.41$, $p<0.001$,, d.f.=8,80) (Figure 2). Culturable soil microbial species richness was significantly greater in plots treated with worm castings compared to all other treatments except for GJ. Treatments without worm castings were not significantly different from the control group.

There was a significant difference in the mean of culturable colony forming units (CFUs) between treatment types ($F=5.003$, $p<0.001$, d.f.=8,80) (Figure 3). Colony forming units were significantly different in plots treated with worm castings compared to control except WC which was not different from the control.

There was a significant difference in the mean Shannon diversity index of culturable microbial species between treatment types ($F=5.70$, $p<0.001$, d.f.=8,80) (Figure 4). WCBO was significantly greater than the control treatment while all other treatments were not different from control.

Soil Water Percent by Weight

There was a significant difference in soil water percent by weight between treatment types ($F=26.62$, $p<0.001$, d.f. =8) (Figure 6). WCBC was significantly greater than all other treatments with 62.60 ± 13.00% water by weight. WCBO and BC were the next highest and were both significantly greater than the control. No other treatments were different than the control group

Microbial Functional Diversity

The average well color development (AWCD) mean measured at 72 hrs across all treatments was 0.6840. This value is the recommended AWCD by Biolog® for continuing with diversity analyses. There was a significant difference in AWCD among treatments ($F=3.541$, $p=0.001$, d.f.=8,80) with WCBC being significantly different from C ($p=0.01$).

There was a significant difference among the mean functional species evenness (E) among different treatment types ($F=5.948$, $p<0.001$, d.f=8, 80) (Figure 7). The mean species evenness of WCBO was significantly greater than the mean functional evenness of C, BO, FH, and BC. BC resulted in the lowest function evenness among treatments.

There was a significant difference in the mean functional Shannon's diversity index among different treatment types ($F=4.671$, $p<0.001$, d.f.=8, 80) (Figure 8). The functional diversity of WCBO was significantly greater than FH, and BC only with no other treatments being different than control.

Soil Organic Matter Carbon

These data were deemed inaccurate due to experimental error. During the absorbance measurement, the last 33 samples consistently showed zero difference between freeze-dried and

non-freeze-dried samples indicating a degradation of absorbance pigment resulting in unreliable measurement.

Soil Temperature

There was no significant difference in mean soil temperature between treatments ($p=0.136$, $F=1.607$, d.f.=8, 80). The general trend was that treatments with worm castings had lower soil temperature than treatments without worm castings (Figure 9).

DISCUSSION

Significant differences in soil microbial diversity, microbial abundance, microbial functional diversity, plant biomass, water holding capacity, and carbon dioxide flux rates among treatment types indicate that the type of soil amendment applied to the soil had a significant effect on these key biological soil health parameters. The largest difference in means between treatments was consistently between WCBC (worm castings and biochar in combination) and the control treatment. WCBC resulted in significantly greater plant biomass, water holding capacity, culturable soil microbial diversity and abundance than all other treatments. WC, WCBO, and WCFH showed significantly higher levels of culturable soil microbial diversity and abundance, water holding capacity, and aboveground plant biomass than the control treatment and other treatments where the worm castings were not present. WCBO resulted in the greatest functional microbial diversity among all treatments. This result was relatively surprising given that WCBC showed greater response in plant biomass and with all other measured variables. This response is fascinating because the bokashi preparation was the simplest to make but still yielded the greatest diversity of nutrient utilization when paired with the worm castings. Further studies should be done to examine this relationship. BC, which was biochar by itself, did not show any

significant difference from the control treatment in any of the test variables measured. Biochar alone is not a source of microbial inoculants, and would therefore not add any potential benefits to the rhizosphere nutrient cycling required for crop responses. However, as shown in WCBC, when biochar is paired with worm castings, this leads to significant responses in microbial and plant growth. Adding all microbial treatments (bokashi, fish hydrolysate, worm castings) to biochar instead of to worm castings should be considered for further studies. Additionally, the positive relationship between culturable microbial diversity, plant biomass, and water holding capacity indicate that these three factors are the ones most significantly affected by the organic soil amendments. Given that above ground plant biomass and soil water holding capacity are the most commonly and easily measured response variables to fertilizer amendments, it should be noted their ability and accuracy in predicting increases in soil microbial diversity. This relationship indicates that soil microbial diversity can be a noteworthy indicator of crop yield and soil health in agricultural systems. The observed increases in microbial functional diversity among plots treated with worm castings indicate that worm castings serve an important role in increasing plant growth through their ability to provide a diverse microbial community capable of cycling more nutrients in the soil than when worm castings were not present. Additionally, the culturable microbial diversity analyses showed that the Shannon's diversity index led to more significant differences than the Simpson's diversity index. This relationship indicates that rare functional groups among microbes responded significantly to the worm casting treatments as the Shannon index is more affected by overall species richness and evenness. It should be noted that measuring diversity in microbial communities using these methods is imperfect because of the vast array of unobserved individuals in the community[81]. Overall, the combination of worm castings and biochar together yielded the most significant differences in all of the major test

variables. This effect is likely due to the combination of stable organic matter carbon substrates in the form of the biochar and worm casting material for the microbes that are native to the worm castings themselves. All of the worm casting treatments showed a stronger response in key metrics like water holding capacity, plant biomass, and microbial diversity, which indicates that the worm castings were a major factor by themselves. All of the treatments when not paired with worm castings did not show significant differences in the key test variables which further emphasizes the importance of the worm castings' presence in the soil for the most significant response.

The findings of this project reflect the common knowledge that increasing soil nutrient sources leads to an increase in plant health and soil microbiome health[82]. Overall, the main factors affected by the soil amendments were the microbial taxonomic and functional diversity, plant growth response, and water holding capacity of the soils. These responses are all interdependent with each other as increasing the available water allows for both the growth of plant matter as well as microbial health in the soil. Additionally, plants are able to release complex nutrients into the rhizosphere as they are healthier and less stressed by a lack of water, which increases the available nutrient sources and interactions among the microbes present and introduced into the soil[36,83]. As these microbial populations are supported by both the availability of water, air, and plant root exudates, they are able to diversify their production of secondary metabolites in a similar way to the plants, leading to increased availability and diversity of nutrients available to the plants and other microbes in the rhizosphere[84]. These three aspects of a thriving rhizosphere have been well documented and describe the basic needs and potential for a more robust and resilient agroecological landscape to form as we begin to feel the effects of climate change and fertilizer shortages due to global trade issues. The effects of introducing

worm castings to the soil were the most profound as those plots treated with worm castings in any combination with fishy hydrolysate, bokashi, and biochar resulted in significantly higher levels of microbial diversity, plant biomass, and water holding capacity than plots that did not receive any worm casting treatments. This effect can possibly be explained by the introduction of more than just complex organic nutrient sources, fulfilling the nutrient needs of the plants, but also the introduction of complex microbial communities. When there is a complex exchange of nutrients between plants and microbes in the rhizosphere, the effects become synergistic with each other and lead to a more dynamic response than if only the plant or microbial community are acted on alone.

The implications outside of academia resulting from this project are potentially substantial. The use of low-cost soil amendments that small-scale and beginning farmers can use to substantially affect the soil microbial communities and therefore the overall performance of their crops is well supported by the results. With the need for more young farmers to enter the agricultural field one of the most important things that they can understand is the importance that a healthy soil ecosystem can play on their success. By continuing to expand on the existing literature available to beginning farmers, who may be starting in suboptimal soil conditions, the tools and resources to help ensure their success can be greatly improved. Additionally, the availability and feasibility of using the soil amendments outlined in this project allows for low-cost implementation by beginning farmers who may not have the capital resources needed to invest in conventional nutrient sources. Not only are these amendments feasible to use, they also have a dramatic effect immediately upon their introduction to the soil. All of the results from this project occurred after a single growing season, indicating that rapid improvements to soil health and plant performance are achievable and likely if applied in a similar manner to the study. The

rapidity of crop performance outcomes is extremely important during the beginning years of starting an agricultural enterprise as that is when the most capital is invested and therefore cash-flow is often tightest during the first few years[85]. One unmeasured but significant response to the worm casting and biochar treatment was that it was the only one to result in any fruit yield from the zucchini. While it is not extremely difficult to grow and see yield from zucchini plants, the fact that none of the other treatments resulted in any fruit is telling that the effects on plant performance resulting from the microbial and soil structure changes previously described is quite significant.

The implications of this project are not exclusive to small and beginning farmers either. Established farmers are seeing increasing costs of fertilizer inputs with reduced efficacy of those nutrients due to the drastic loss of topsoil occurring throughout the modern agricultural world[86,87]. As topsoil and organic matter are degraded across agricultural fields, resilience and crop performance also decrease. These effects can be counteracted to some extent using no-till methods, but while this practice can slow down topsoil loss, it does not stop or reverse its loss[88]. By introducing complex organic soil amendments to degraded soils in agricultural land, the microbial community and therefore plant performance response are likely to respond favorably. While it may not be completely feasible to amend the soil at the scale that was done in this study, slow, incremental increases to the complex structure of the soil may still result in increased microbial community diversity and plant health factors. Further studies exploring these same treatments on more established agricultural fields and different parent soil structures may show similar results.

While the scope of this project was broad, some issues arose with regards to feasibility and funding. This being a novel project with no previous studies in this site meant that many

baseline measurements were needed. Further studies using a similar approach at the site could lead to greater detail into the intricacies of the plant-microbe interactions occurring with these soil amendment applications. For instance, the response of the microbial community structure was limited to culturable diversity and functional diversity. While these two factors can elucidate a significant portion of the overall microbial community composition, they fall short in understanding what specific microbes are present and active in the rhizosphere. Incorporating a metagenomic analysis using next-gen sequencing techniques would provide added details on what specific microbes were present in the soil. Additionally, rare and fastidious microbes that do not grow well on agar plates outside of the soil structure could be observed. Quite often, those rare species carry significant weight in the function and health of an ecosystem[89]. Compound the genetic diversity of bacteria in the soil with an analysis of fungal and mesofaunal diversity and a much more complete picture of the rhizosphere comes into view. Fungal community structure would be extremely interesting to study next because of the profound impact that fungi have on the nutrient cycling and health of plant communities[90]. Contrasting the effects of a more robust microbial community diversity analysis with measurements of the plants' physicochemical response could be another significant response to the different soil amendments used. If indeed, the microbial community diversity responding in a positive manner to the soil amendments results in an increase in the overall increase in the nutrient cycling then there should be a complimentary effect on the metabolic functioning of the plants.

The soil organic matter content and CO_2 flux rates did not show significant changes between treatment types. This is likely a factor of time as these variables are indicators of longer-term carbon dynamics in a soil ecosystem. The timeframe of this study did not allow for these variables' responses to be fully evident, so continuous study of these factors may lead to

more observed effects. However, these patterns did not reflect the other, shorter term variables measured. Additionally, the potassium dichromate – sulfuric acid method for extracting and measuring soil organic matter carbon did not result in any coherent results. This indicates that there was a faulty procedure which resulted in inconclusive results, possibly due to the length of time between rapid oxidation of the organic carbon and absorbance measurements. Unfortunately, soil organic matter carbon is commonly regarded as one of the most indicative factors when assessing soil health and often shows a strong positive relationship to plant health, microbial diversity, water holding capacity, and overall good soil structure. Further studies with more funding and better access to lab resources should be conducted to further explore the effects of these soil amendments on soil health and efficacy of remediating degraded soils in general.

In conclusion, the addition of worm castings as a stable, complex form of organic matter and microbial inoculum had a positive effect on crop yield in a previously degraded soil in the first year of application. Furthermore, addition of biochar to the worm casting treatment did indicate an even greater impact on soil and plant health than with worm castings alone.

Table 1: Treatment number and organic amendment(s) used in each corresponding group.

Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7	Treatment 8	Treatment 9
Control	Worm Castings	Worm Castings + Bokashi Tea	Worm Castings + Fish Hydrolysate	Worm Castings + Biochar	Bokashi Tea	Fish Hydrolysate	Biochar	Grow Juice 2

Table 2: Descriptive statistics summary of dependent variables. Significance codes based on Tukey HSD posthoc test after ANOVA analysis signifying significant different means among treatments. If no letters are shown by a given value then no significant difference between means was observed.

	Treatment 1 (Control)	Treatment 2 (Worm Castings)	Treatment 3 (Worm Castings & Bokashi Tea)	Treatment 4 (Worm Castings & Fish Hydrolysate)	Treatment 5 (Worm Castings & Biochar)	Treatment 6 (Bokashi Tea)	Treatment 7 (Fish Hydrolysate)	Treatment 8 (Biochar)	Treatment 9 (Grow Juice 2)
Culturable Simpson's Diversity Index	0.59 ± 0.28 a	0.75 ± 0.14 ab	0.77 ± 0.08 a	0.76 ± 0.13 ab	0.72 ± 0.11 abc	0.72 ± 0.11 abc	0.69 ± 0.12 bc	0.78 ± 0.12 abc	0.74 ± 0.09 abc
Culturable Shannon's Diversity Index	1.06 ± 0.48 a	1.57 ± 0.39 a, b	1.67 ± 0.19 b	1.53 ± 0.40 a, b	1.44 ± 0.35 a, b	1.41 ± 0.36 a, b	1.19 ± 0.33 a,b	1.35 ± 0.50 a, b	1.32 ± 0.28 a, b
Culturable Species Evennes	0.69 ± 0.14	0.77 ± 0.88	0.75 ± 0.07	0.71 ± 0.17	0.73 ± 0.08	0.74 ± 0.09	0.74 ± 0.09	0.78 ± 0.10	0.68 ± 0.06
Colony Forming Units	18.30 ± 8.82 c	36.50 ± 20.32 abc	50.20 ± 16.75 b	38.20 ± 14.97 ab	40.10 ± 17.49 ab	38.00 ± 16.98 abc	28.80 ± 15.42 abc	26.80 ± 14.30 ac	37.44 ± 17.59 abc
Soil Temp (°C)	25.10 ± 1.86	24.70 ± 1.70	24.30 ± 1.42	24.20 ± 1.75	23.70 ± 1.49	25.40 ± 1.73	25.10 ± 1.10	25.10 ± 2.08	25.78 ± 1.39
CO2 Flux	-1.85 ± 0.07	-1.86 ± 0.06	-1.88 ± 0.09	-1.84 ± 0.11	-1.82 ± 0.10	-1.83 ± 0.05	-1.86 ± 0.09	-1.86 ± 0.05	-1.93 ± 0.13
Plant Biomass (g)	8.15 ± 6.66 c	31.81 ± 20.58 ab	26.18 ± 18.70 abc	22.50 ± 15.82 abc	39.54 ± 21.78 a	7.20 ± 9.61 c	12.50 ± 15.51 c	10.78 ± 10.83 c	9.55 ± 8.63 c
Soil Water Percent by Weight	10.80 ± 7.40% d	25.40 ± 12.80% bcd	32.20 ± 12.60 b	18.80 ± 12.00% bcd	64.60 ± 13.00% a	10.80 ± 6.80 d	13.00 ± 6.20% cd	26.60 ± 14.00 bc	10.00 ± 5.80 d
Functional Richness	29.5 ± 1.51	28.3 ± 2.54	28.3 ± 1.95	27.7 ± 3.53	29.3 ± 1.57	28.1 ± 2.73	28.1 ± 3.64	28.3 ± 2.67	29.11 ± 2.32
Funcitonal Diversity Shannon's Diversity Index (H)	2.91 ± 0.12 abc	2.97 ± 0.14 bc	3.06 ± 0.08 c	2.91 ± 0.15 abc	3.02 ± 0.11 bc	2.88 ± 0.14 abc	2.85 ± 0.17 ab	2.77 ± 0.12 a	2.93 ± 0.10 abc
Functional Evenneess	0.86 ± 0.03 ab	0.89 ± 0.02 bc	0.92 ± 0.02 c	0.88 ± 0.03 bc	0.90 ± 0.02 bc	0.87 ± 0.04 ab	0.86 ± 0.4 ab	0.83 ± 0.05 a	0.87 ± 0.03 abc

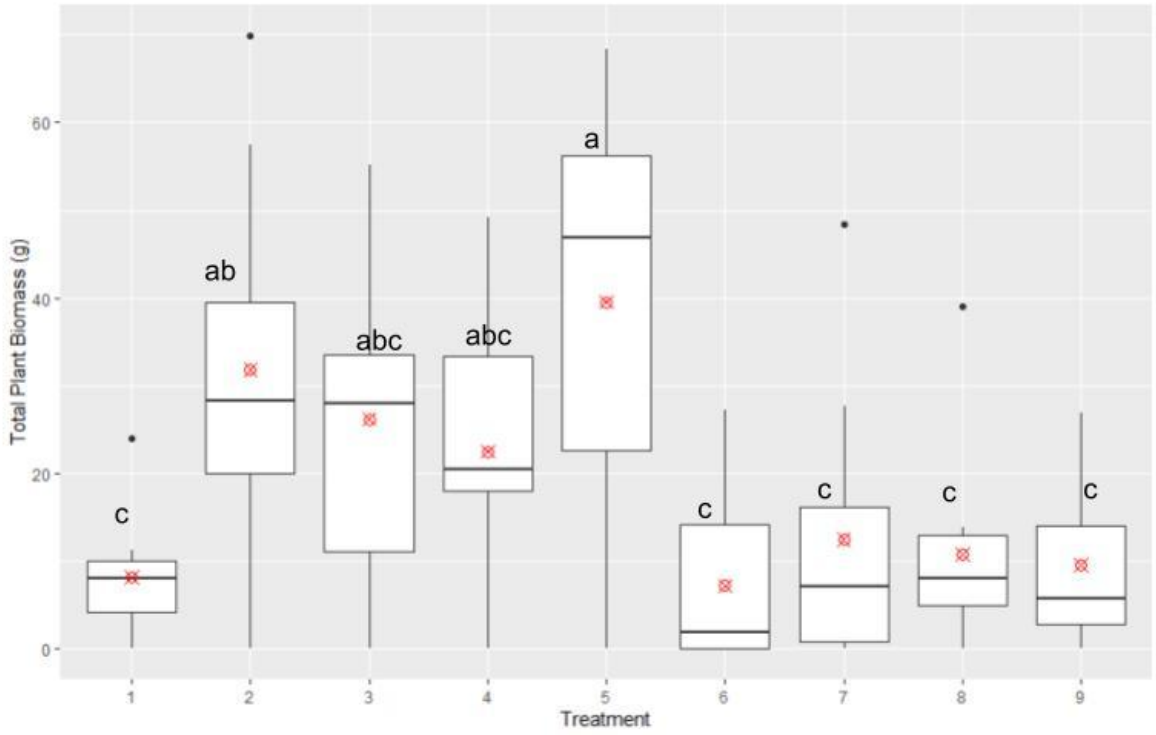

Figure 1: Total plant biomass (g) by treatment type. WC and WCBC were significantly greater than C, BO, FH, BC, and GJ. Treatment 1 = control. Treatment 2 = worm castings only. Treatments 3 = worm castings + bokashi, Treatment 4 = worm castings + fish hydrolysate, Treatment 5 = worm castings + biochar. Treatment 6 = bokashi alone, Treatment 7 = fish hydrolysate alone, Treatment 8 = biochar alone, and Treatment 9 = Grow Juice 2 alone. The line in the box plot signifies median while the red dot signifies the mean.

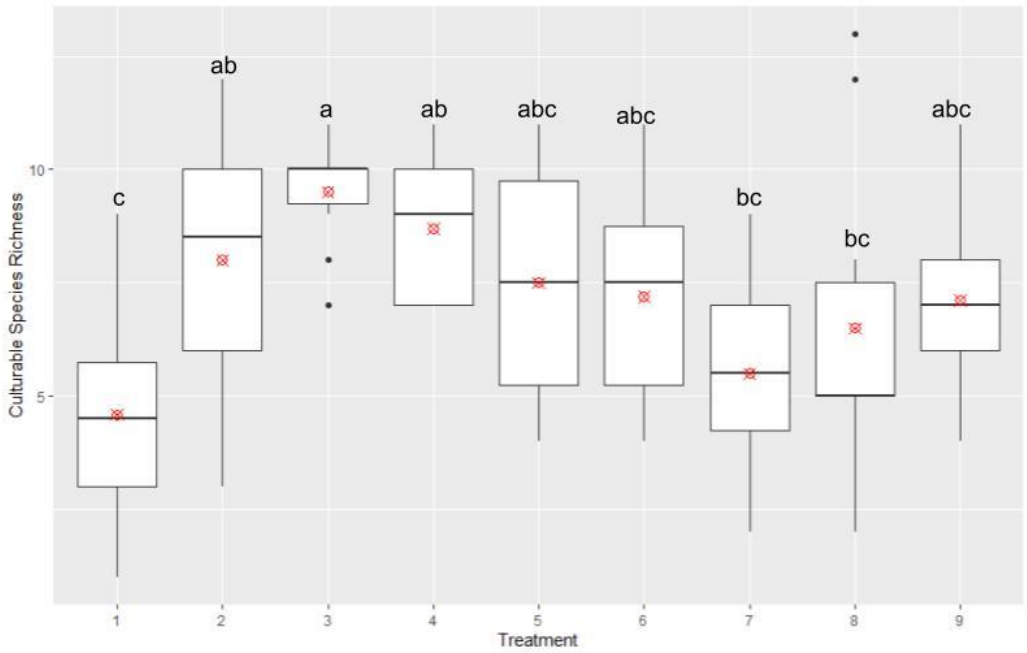

Figure 2: Culturable microbial species richness. WC, WCBO, and WCFH were significantly different than C. WCBO was significantly different than FH, and BC. Treatment 1 = control. Treatment 2 = worm castings only. Treatments 3 = worm castings + bokashi, Treatment 4 = worm castings + fish hydrolysate, Treatment 5 = worm castings + biochar. Treatment 6 = bokashi alone, Treatment 7 = fish hydrolysate alone, Treatment 8 = biochar alone, and Treatment 9 = Grow Juice 2 alone. The line in the box plot signifies median while the red dot signifies the mean.

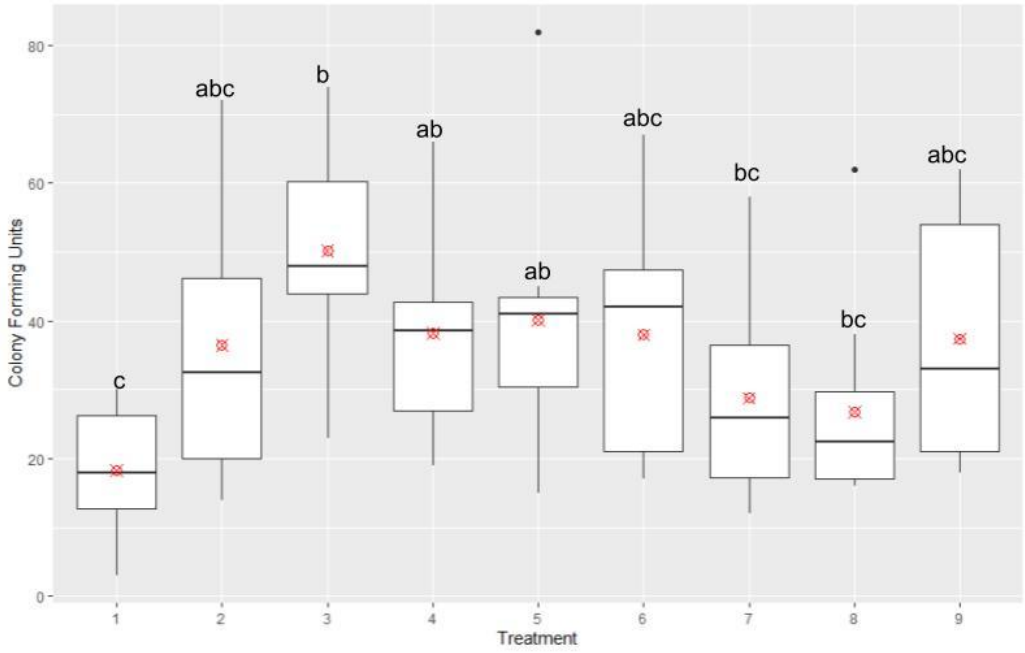

Figure 3: Culturable colony forming units of soil bacteria were significantly different between treatments. WCBO, WCFH, and WCBC were significantly different from C. Treatment 1 = control. Treatment 2 = worm castings only. Treatments 3 = worm castings + bokashi, Treatment 4 = worm castings + fish hydrolysate, Treatment 5 = worm castings + biochar. Treatment 6 = bokashi alone, Treatment 7 = fish hydrolysate alone, Treatment 8 = biochar alone, and Treatment 9 = Grow Juice 2 alone. The line in the box plot signifies median while the red dot signifies the mean.

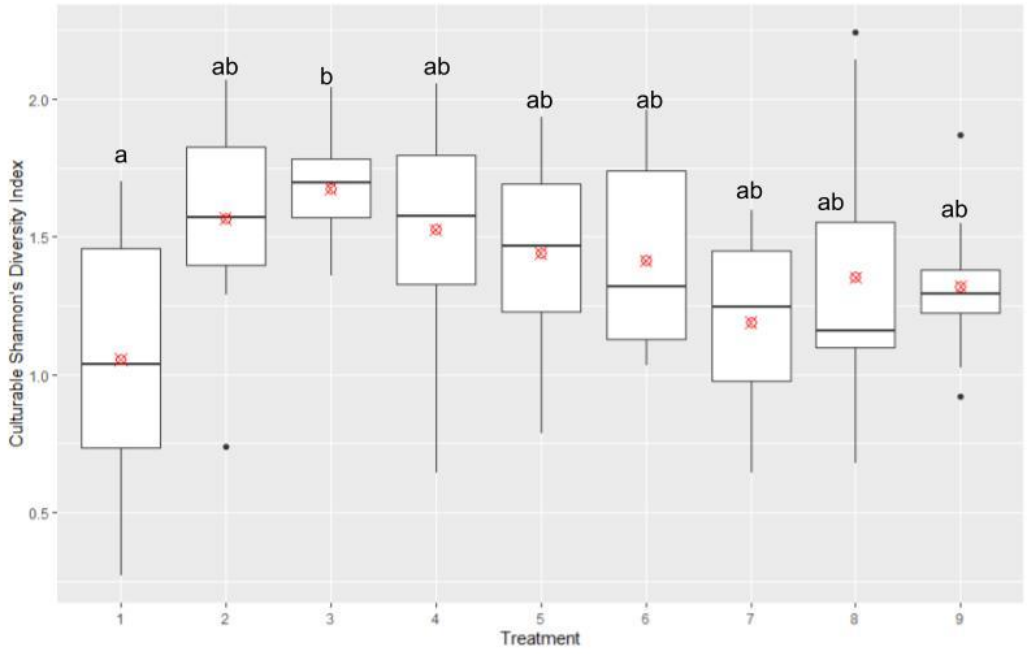

Figure 4: Culturable microbial species diversity calculated using the Shannon -Weiner diversity index (H). WCBO was significantly different than C. Treatment 1 = control. Treatment 2 = worm castings only. Treatments 3 = worm castings + bokashi, Treatment 4 = worm castings + fish hydrolysate, Treatment 5 = worm castings + biochar. Treatment 6 = bokashi alone, Treatment 7 = fish hydrolysate alone, Treatment 8 = biochar alone, and Treatment 9 = Grow Juice 2 alone. The line in the box plot signifies median while the red dot signifies the mean.

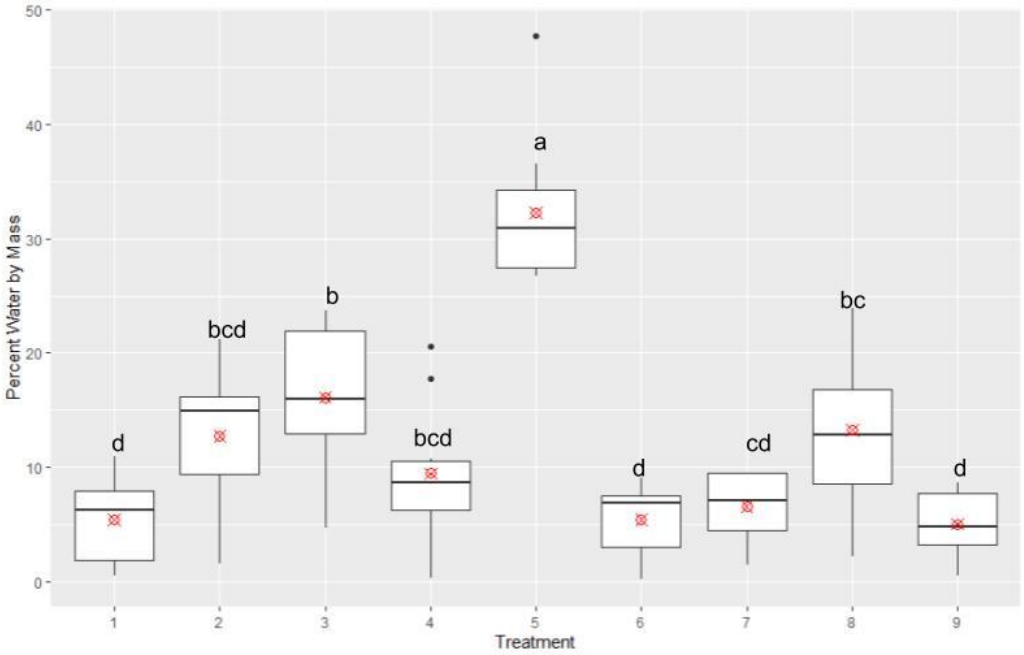

Figure 5: Total water mass as percent of total soil mass of 15 g soil samples between treatments. WCBC was significantly different from all other treatments. C was significantly different from treatments WC, WCBO, WCBC, BC. Treatment 1 = control. Treatment 2 = worm castings only. Treatments 3 = worm castings + bokashi, Treatment 4 = worm castings + fish hydrolysate, Treatment 5 = worm castings + biochar. Treatment 6 = bokashi alone, Treatment 7 = fish hydrolysate alone, Treatment 8 = biochar alone, and Treatment 9 = Grow Juice 2 alone. The line in the box plot signifies median while the red dot signifies the mean.

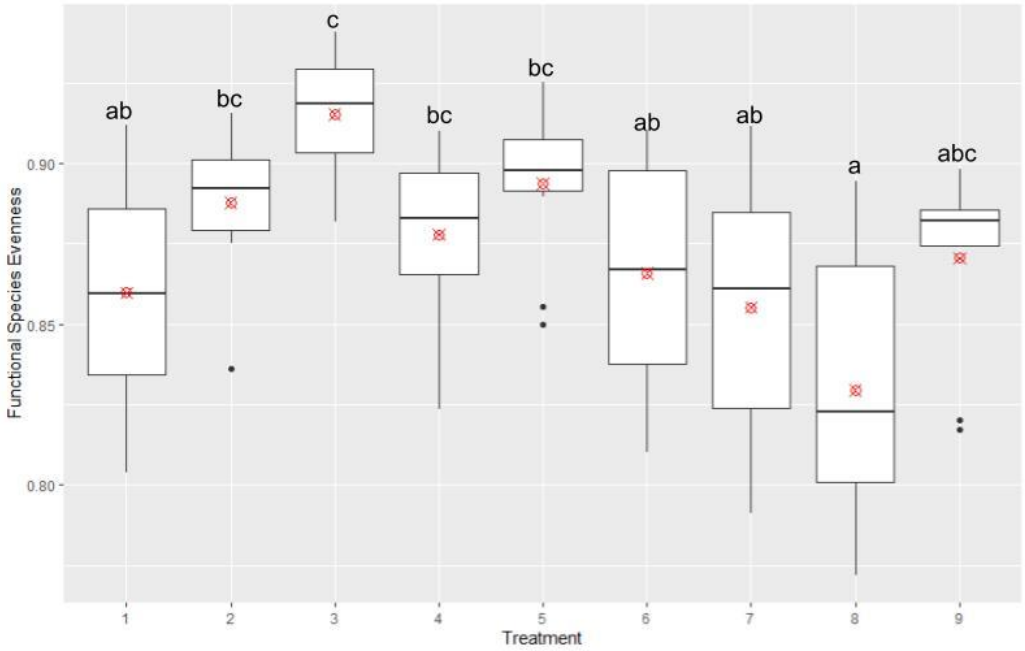

Figure 6: Means of microbial functional evenness. WCBO had the highest species evenness value and was significantly different from treatments C, WCFH, BO, FH, BC, GJ. Worm casting treatments were significantly different from all treatments without worm castings except for GJ. Treatment 1 = control. Treatment 2 = worm castings only. Treatments 3 = worm castings + bokashi, Treatment 4 = worm castings + fish hydrolysate, Treatment 5 = worm castings + biochar. Treatment 6 = bokashi alone, Treatment 7 = fish hydrolysate alone, Treatment 8 = biochar alone, and Treatment 9 = Grow Juice 2 alone. The line in the box plot signifies median while the red dot signifies the mean.

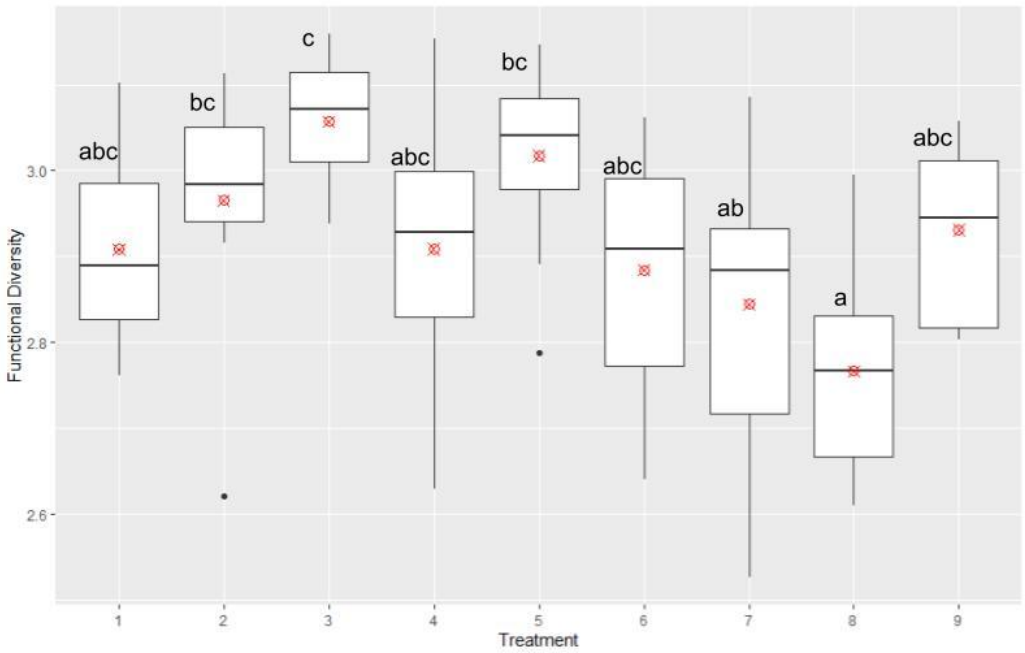

Figure 7: Mean functional microbial Shannon diversity index values. WC, WCBO, WCBC were significantly different from BC. WCBO was also significantly different from BO, FH. Treatment 1 = control. Treatment 2 = worm castings only. Treatments 3 = worm castings + bokashi, Treatment 4 = worm castings + fish hydrolysate, Treatment 5 = worm castings + biochar. Treatment 6 = bokashi alone, Treatment 7 = fish hydrolysate alone, Treatment 8 = biochar alone, and Treatment 9 = Grow Juice 2 alone. The line in the box plot signifies median while the red dot signifies the mean.

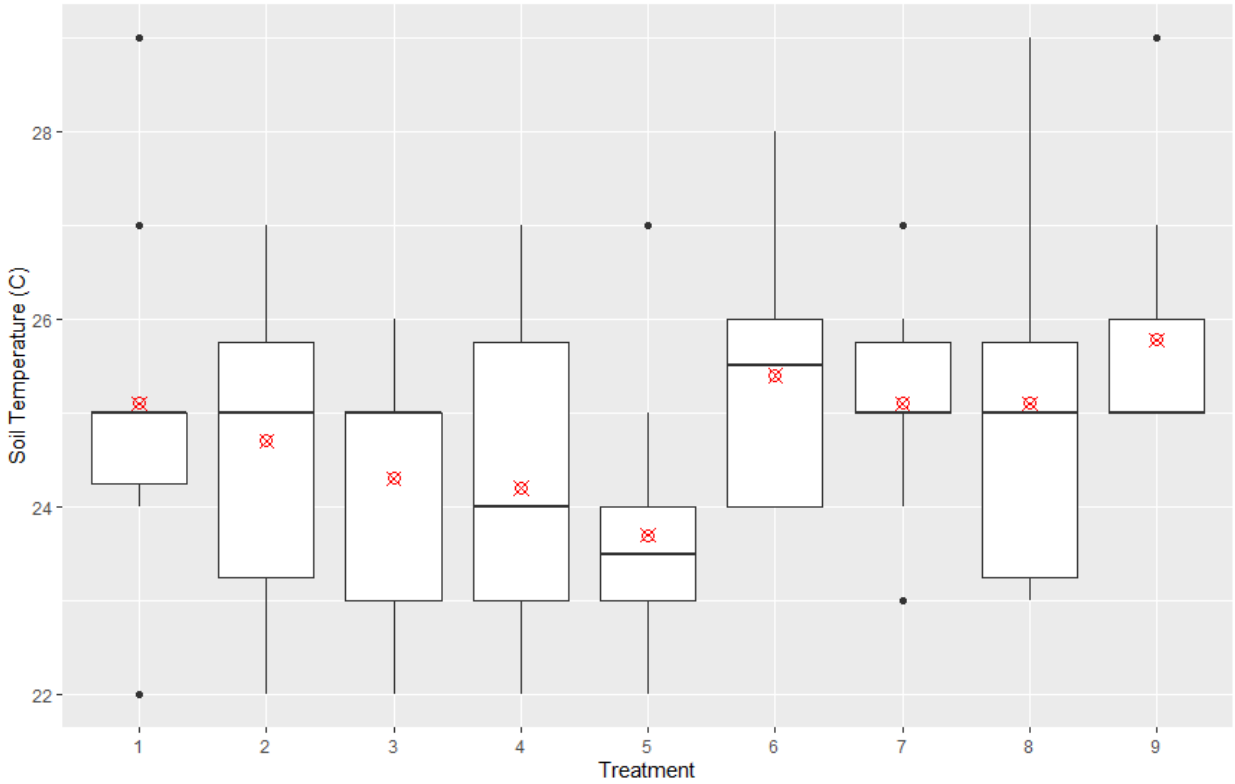

Figure 8: Soil temperature (°C) in all treatments. No significant differences in temperature were observed. Treatment 1 = control. Treatment 2 = worm castings only. Treatments 3 = worm castings + bokashi, Treatment 4 = worm castings + fish hydrolysate, Treatment 5 = worm castings + biochar. Treatment 6 = bokashi alone, Treatment 7 = fish hydrolysate alone, Treatment 8 = biochar alone, and Treatment 9 = Grow Juice 2 alone. The line in the box plot signifies median while the red dot signifies the mean.

LITERATURE CITED

1. Kheyrodin, H., Ghazvinian, K. & Taherian, M. Tillage and manure effect on soil microbial biomass and respiration, and on enzyme activities. *Afr. J. Biotechnol.* **11**, 14652–14659 (2012).
2. U.S. Department of Agriculture. 2020. Summary Report: 2017 National Resources Inventory, Natural Resources Conservation Service, Washington, DC, and Center for Survey Statistics and Methodology, Iowa State University, Ames, Iowa.
3. You, G. C. N., Mark W. Rosegrant, Amanda Palazzo, Ian Gray, Christina Ingersoll, Richard Robertson, Simla Tokgoz, Tingju Zhu, Timothy B. Sulser, Claudia Ringler, Siwa Msangi, and Liangzhi. *Food Security, Farming, and Climate Change to 2050: Scenarios, Results, Policy Options*. (Intl Food Policy Res Inst, 2010).
4. Drewnowski, A. Concept of a nutritious food: toward a nutrient density score. *Am. J. Clin. Nutr.* **82**, 721–732 (2005).
5. Larsen, K. & Gilliland, J. A farmers' market in a food desert: Evaluating impacts on the price and availability of healthy food. *Health Place* **15**, 1158–1162 (2009).
6. Wortman, S. E. & Lovell, S. T. Environmental Challenges Threatening the Growth of Urban Agriculture in the United States. *J. Environ. Qual.* **42**, 1283–1294 (2013).
7. Secretary General, U. N. *The United Nations Secretary-General's High-Level Task force on Global Food and Nutrition Security*. http://www.un.org/en/issues/food/taskforce/pdf/ZHC%20ANs-%20All%20Merged%20Rev%20May%202016.pdf (2015).
8. Wheeler, T. & Braun, J. von. Climate Change Impacts on Global Food Security. *Science* **341**, 508–513 (2013).
9. Schrama, M., de Haan, J. J., Kroonen, M., Verstegen, H. & Van der Putten, W. H. Crop yield

gap and stability in organic and conventional farming systems. *Agric. Ecosyst. Environ.* **256**, 123–130 (2018).

10. Schader, C., Stolze, M. & Gattinger, A. Environmental performance of organic farming. in *Green Technologies in Food Production and Processing*.183–210 (2012).
11. Bengtsson, J., Ahnström, J. & Weibull, A.-C. The effects of organic agriculture on biodiversity and abundance: a meta-analysis. *J. Appl. Ecol.* **42**, 261–269 (2005).
12. Condron, L. M. *et al.* A comparison of soil and environmental quality under organic and conventional farming systems in New Zealand. *N. Z. J. Agric. Res.* **43**, 443–466 (2000).
13. Badgley, C., Moghtader, J., Quintero, E., Zakem, E., Chappell, M., Avilés-Vázquez, K., . . . Perfecto, I. (2007). Organic agriculture and the global food supply. *Renewable Agriculture and Food Systems, 22*(2), 86-108. doi:10.1017/S1742170507001640
14. Alonso-Fradejas, A., *et al. Contextualizing Food Sovereignty: The politics of convergence among movements in the US, Third World Quarterly, Food First.* **36.** 3, 618-635 (2015).
15. Alonso-Fradejas, A., *et al.* Food Sovereignty: Convergence and contradictions, conditions and challenges. *Food First - Third World Quarterly.* **36:3,** 431-448 (2015)
16. Pimental, D., *et al.* Environmental and Economic Costs of Soil Erosion and Conservation Benefits. *Science*. **267.** 1117-1123. (1995).
17. Ferris, H., Bongers, T. & de Goede, R. G. M. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Appl. Soil Ecol.* **18**, 13–29 (2001).
18. Kleber, M. *et al.* Old and stable soil organic matter is not necessarily chemically recalcitrant: implications for modeling concepts and temperature sensitivity. *Glob. Change Biol.* **17**, 1097–1107 (2011).
19. Abawi, G. S. & Widmer, T. L. Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. *Appl. Soil Ecol.* **15**, 37–47 (2000).

20. Worthington, V. Nutritional Quality of Organic Versus Conventional Fruits, Vegetables, and Grains | The Journal of Alternative and Complementary Medicine. *The Journal of ALternative and Complementary Medicine*. **7:2,** (2004)
21. Altieri, M. A., Funes-Monzote, F. R. & Petersen, P. Agroecologically efficient agricultural systems for smallholder farmers: contributions to food sovereignty. *Agron. Sustain. Dev.* **32**, 1–13 (2012).
22. Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R. & Polasky, S. Agricultural sustainability and intensive production practices. *Nature* **418**, 671–677 (2002).
23. Lammerts van Bueren, E. T., Struik, P. C. & Jacobsen, E. Ecological concepts in organic farming and their consequences for an organic crop ideotype. *NJAS - Wagening. J. Life Sci.* **50**, 1–26 (2002).
24. Fernandez, A. L. *et al.* Associations between soil bacterial community structure and nutrient cycling functions in long-term organic farm soils following cover crop and organic fertilizer amendment. *Sci. Total Environ.* **566–567**, 949–959 (2016).
25. Bulluck, L. R., Brosius, M., Evanylo, G. K. & Ristaino, J. B. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. *Appl. Soil Ecol.* **19**, 147–160 (2002).
26. Marschner, P., Kandeler, E. & Marschner, B. Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biol. Biochem.* **35**, 453–461 (2003).
27. Cattle, S. R. & Quilty, J. R. Use and understanding of organic amendments in Australian agriculture: a review. *Soil Res.* **49**, 1+ (2011).
28. Diacono, M. & Montemurro, F. Long-Term Effects of Organic Amendments on Soil Fertility. in *Sustainable Agriculture Volume 2* (eds. Lichtfouse, E., Hamelin, M., Navarrete, M. & Debaeke, P.) 761–786 (Springer Netherlands, 2011). doi:10.1007/978-94-007-0394-0_34.
29. Frey, S. D., Knorr, M., Parrent, J. L. & Simpson, R. T. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine

forests. *For. Ecol. Manag.* **196**, 159–171 (2004).

30. de Mazancourt, C. *et al.* Predicting ecosystem stability from community composition and biodiversity. *Ecol. Lett.* **16**, 617–625 (2013).
31. Berendsen, R. L., Pieterse, C. M. J. & Bakker, P. A. H. M. The rhizosphere microbiome and plant health. *Trends Plant Sci.* **17**, 478–486 (2012).
32. Yang, J., Kloepper, J. W. & Ryu, C.-M. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci.* **14**, 1–4 (2009).
33. Ryan, M. & Ash, J. Effects of phosphorus and nitrogen on growth of pasture plants and VAM fungi in SE Australian soils with contrasting fertiliser histories (conventional and biodynamic). *Agric. Ecosyst. Environ.* **73**, 51–62 (1999).
34. Bot, A. & Benites, J. *The Importance of Soil Organic Matter: Key to Drought-resistant Soil and Sustained Food Production*. (Food & Agriculture Org., 2005).
35. Lorenz, K. & Kandeler, E. Biochemical characterization of urban soil profiles from Stuttgart, Germany. *Soil Biol. Biochem.* **37**, 1373–1385 (2005).
36. Zhang, C. & Anderson, A. J. The Gluconeogenic Pathway in a Soil Mycobacterium Isolate with Bioremediation Ability. *Curr. Microbiol.* **66**, 122–131 (2012).
37. Courchesne, F., Cloutier-Hurteau, B. & Turmel, M.-C. Relevance of Rhizosphere Research to the Ecological Risk Assessment of Trace Metals in Soils. *Hum. Ecol. Risk Assess. Int. J.* **14**, 54–72 (2008).
38. Lutts, S. & Lefèvre, I. How can we take advantage of halophyte properties to cope with heavy metal toxicity in salt-affected areas? *Ann. Bot.* **115**, 509–528 (2015).
39. Degens, B. P., Schipper, L. A., Sparling, G. P. & Duncan, L. C. Is the microbial community in a soil with reduced catabolic diversity less resistant to stress or disturbance? *Soil Biol. Biochem.* **33**, 1143–1153 (2001).
40. Kaufman, J. L. & Bailkey, M. *Farming inside cities: Entrepreneurial urban agriculture in the United States*. (Lincoln Institute of Land Policy Cambridge, MA, 2000).

41. Selden, P., DuPonte, M., Sipes, B. & Dinges, K. Small-Scale Vermicomposting. 4 (2005).
42. Lehmann, J. & Joseph, S. *Biochar for Environmental Management: Science and Technology*. (Routledge, 2012).
43. Vasileva-Tonkova, E., Nustorova, M. & Gushterova, A. New Protein Hydrolysates from Collagen Wastes Used as Peptone for Bacterial Growth. *Curr. Microbiol.* **54**, 54–57 (2006).
44. Krishnamoorthy, R. V. & Vajranabhaiah, S. N. Biological activity of earthworm casts: An assessment of plant growth promotor levels in the casts. *Proc. Anim. Sci.* **95**, 341–351 (1986).
45. Drake, H. L. & Horn, M. A. As the Worm Turns: The Earthworm Gut as a Transient Habitat for Soil Microbial Biomes. *Annu. Rev. Microbiol.* **61**, 169–189 (2007).
46. Blouin, M. *et al.* A review of earthworm impact on soil function and ecosystem services. *Eur. J. Soil Sci.* **64**, 161–182 (2013).
47. Pathma, J. & Sakthivel, N. Microbial diversity of vermicompost bacteria that exhibit useful agricultural traits and waste management potential. 19 (2012).
48. Lima, H., *et al.* Pedogenesis and pre-Colombian land use of “Terra Preta Anthrosols” (“Indian black earth”) of Western Amazonia. *Geoderma.* **110,** 1-17. (2002)
49. Olson, G. W. Archaeology: Lessons on future soil use. *J. Soil Water Conserv.* **36**, 261–264 (1981).
50. Lehmann, J. *et al.* Biochar effects on soil biota – A review. *Soil Biol. Biochem.* **43**, 1812–1836 (2011).
51. Jaiswal, A., *et al.* Activating biochar by manipulating the bacterial and fungal microbiome through pre-conditioning. *New Phytologist.* **219,** 363-377 (2018)
52. Gevaert, B., Veryser, L., Verbeke, F., Wynendaele, E. & De Spiegeleer, B. Fish Hydrolysates: A Regulatory Perspective of Bioactive Peptides. *Protein Pept. Lett.* **23**, 1052–1060 (2016).
53. Kristinsson, H. G. & Rasco, B. A. Fish Protein Hydrolysates: Production, Biochemical, and

Functional Properties. *Crit. Rev. Food Sci. Nutr.* **40**, 43–81 (2000).

54. He, S., Franco, C. & Zhang, W. Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). *Food Res. Int.* **50**, 289–297 (2013).
55. Vilamil, O., *et al.* Fish viscera protein hydrolysates: Production, potential applications and functional and bioactive properties. *Food Chemistry.* **224,** 160-171 (2017)
56. Petrova, I., *et al.* Production of fish protein hydrolysates step by step: technological aspects, equipment used, major energy costs and methods of their. *International Aquatic Research.* ***10,*** *223-241 (2018)*
57. Ahuja, I., *et al.* Fish and fish waste-based fertilizers in organic farming – With status in Norway: A review. *Waste Management.* **115,** 95-112 (2020).
58. Close, D. C., Beadle, C. L. & Brown, P. H. The physiological basis of containerised tree seedling 'transplant shock': a review. *Aust. For.* **68**, 112–120 (2005).
59. Doyle, C., Pellow, B., Rapmund, R. & Ooi, M. Preparing threatened plants for translocation: does home soil addition and nutrient loading improve growth and flowering? *Plant Ecol.* **222**, (2021).
60. Dufault, R., Influence of Nutritional Conditioning on Muskmelon Transplant Quality and Early Yield in: *Journal of the American Society for Horticultural Science.* **111,** (1986)
61. Rahman, K. M. A. & Zhang, D. Effects of Fertilizer Broadcasting on the Excessive Use of Inorganic Fertilizers and Environmental Sustainability. *Sustainability* **10**, 759 (2018).
62. Kulkarni, S. & Goswami, A. Effect of Excess Fertilizers and Nutrients: A Review on Impact on Plants and Human Population. *Proceedings of INternation Conference on Sustainable Computing in Science, Technology and Management (SUSCOM), Amity University. Rajasthan, Jaipur - India* (2019).
63. Li, Z. *et al.* Excessive Nitrogen Fertilization Favors the Colonization, Survival, and Development of Sogatella furcifera via Bottom-Up Effects. *Plants* **10**, 875 (2021).
64. Mozafar, A. Nitrogen fertilizers and the amount of vitamins in plants: A review. *J. Plant Nutr.*

16, 2479–2506 (1993).

65. Elhanafi, L. *et al.* Impact of Excessive Nitrogen Fertilization on the Biochemical Quality, Phenolic Compounds, and Antioxidant Power of *Sesamum indicum* L Seeds. *J. Food Qual.* **2019**, e9428092 (2019).
66. Yoder, N. & Davis, J. G. Organic Fertilizer Comparison on Growth and Nutrient Content of Three Kale Cultivars. *HortTechnology* **30**, 176–184 (2020).
67. Cram, W., Pinocytosis in Plants. *New Phytologist. **84,** 1-17 (1980)*
68. Blackhall, N. W., Finch, R. P., Power, J. B., Cocking, E. C. & Davey, M. R. Flow cytometric quantification of electroporation-mediated uptake of macromolecules into plant protoplasts. *Protoplasma* **186**, 50–56 (1995).
69. Sadowska, U., Domagała-Świątkiewicz, I. & Żabiński, A. Biochar and Its Effects on Plant–Soil Macronutrient Cycling during a Three-Year Field Trial on Sandy Soil with Peppermint (Mentha piperita L.). Part I: Yield and Macro Element Content in Soil and Plant Biomass. *Agronomy* **10**, 1950 (2020).
70. Herencia, J. F. *et al.* Comparison between Organic and Mineral Fertilization for Soil Fertility Levels, Crop Macronutrient Concentrations, and Yield. *Agron. J.* **99**, 973–983 (2007).
71. Footer, A. *Bokashi Composting: Scraps to Soil in Weeks*. (New Society Publishers, 2013).
72. Quiroz, M., Céspedes, C., Bokashi as an Amendment and Source of Nitrogen in Sustainable Agricultural Systems: a Review. *Journal of Soil Science and Plant Nutrition.* **19,** 237-248 (2019)
73. Shin, K., van Diepen, G., Blok, W. & van Bruggen, Ariena. H. C. Variability of Effective Micro-organisms (EM) in bokashi and soil and effects on soil-borne plant pathogens. *Crop Prot.* **99**, 168–176 (2017).
74. Gaggia, F., *et al.* Inoculation with microorganisms of Lolium perenne L.: evaluation of plant growth parameters and endophytic colonization of roots. *New Biotechnology. **30**, 695-704 (2013)*

75. Chontal, M. A. H. *et al.* Nutrient content of fermented fertilizers and its efficacy in combination with hydrogel in Zea mays L. *Int. J. Recycl. Org. Waste Agric.* **8**, 309–315 (2019).
76. Merfield, C. N., Chamberlain, T. P. & Daly Mike, J. Utilisation of effective microorganisms commercial organic agriculture: a case study from New Zealand. (1997).
77. Wang, J., *et al.* Effect of microbial inoculation on physicochemical properties and bacterial community structure of citrus peel composting. *Bioresource Technology.* **291**, 121843 (2019)
78. Noh, N. J. *et al.* Temperature responses of carbon dioxide fluxes from coarse dead wood in a black ash wetland. *Wetl. Ecol. Manag.* **27**, 157–170 (2019).
79. Baker, J. M. Measurement of soil water content. *Remote Sens. Rev.* **5**, 263–279 (1990).
80. Ridout, M., Serial Dilution Assay. *Encyclopedia of Biostatistics.*
81. Haegeman, B. *et al.* Robust estimation of microbial diversity in theory and in practice. *ISME J.* **7**, 1092–1101 (2013).
82. Thonart, P., Nihorimbere*, V., Ongena*, M. & Smargiassi, M. Beneficial effect of the rhizosphere microbial community for plant growth and health. *Base* (2011).
83. Cheynier, V., Comte, G., Davies, K. M., Lattanzio, V. & Martens, S. Plant phenolics: Recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiol. Biochem.* **72**, 1–20 (2013).
84. Marulanda, A., Barea, J.-M. & Azcón, R. Stimulation of Plant Growth and Drought Tolerance by Native Microorganisms (AM Fungi and Bacteria) from Dry Environments: Mechanisms Related to Bacterial Effectiveness. *J. Plant Growth Regul.* **28**, 115–124 (2009).
85. Paloschi, L., 'An Exploration of the Role of Farm Business Decisions in Addressing Fi'. *Master of Science in Agriculture Thesis - Missouri State University.* (2021).
86. Hafner, S., Is fertilization efficiency misleading? *Nature.* **422**, 397-398 (2003)
87. Al-Kaisi, M. Soil erosion and crop productivity: topsoil thickness. *Soil Eros. Crop Product.* 5.
88. Powlson, D., *et al.,* Limited potential of no-till agriculture for climate change mitigation.

Nature Climate Change. **4**, 678-683 (2014)

89. Wu, Y., *et al.* Rare soil microbial taxa regulate the negative effects of land degradation drivers on soil organic matter decomposition. *Journal of Applied Ecology*. **58**, 1658-1669 (2021)
90. Strekenbur, E., *et al.* Changes in fungal communities along a boreal forest soil fertility gradient. *New Phytologist.* **207**, 1145-1158 (2015)

APPENDIX

R Packages and Versions		
Package	Description	Version
AICcmodavg	Model Selection and Multimodel Inference Based on (Q)AIC(c)	2.3-1
base	The R Base Package	4-2.1
broom	Convert Statistical Objects into Tidy Tables	1.01
data.table	Extension of 'data.frame'	1.14.2
datasets	The R Datasets Package	4.2.1
dplyr	A Grammar of Data Manipulation	1.0.10
forcats	Tools for Working with Categorical Variables (Factors	0.5.2
FSA	Simple Fisheries Stock Assessment Methods	0.9.3
ggfortify	Data Visualization Tools for Statistical Analysis Results	0.4.14
ggplot2	Create Elegant Data Visualisations Using the Grammar of Graphics	3.3.6
ggpubr	ggplot2' Based Publication Ready Plots	0.4.0
graphics	The R Graphics Package	4.2.1
grDevices	The R Graphics Devices and Support for Colours and Fonts	4.2.1
haven	Import and Export 'SPSS', 'Stata', and 'SAS' Files	2.5.1
methods	Formal Methods and Classes	4.2.1
purrr	Functional Programming Tools	0.3.4
readr	Read Rectangular Text Data	2.1.2
readxl	Read Excel Files	1.4.1
stats	The R Stats Package	4.2.1
stringr	Simple, Consistent Wrappers for Common String Operations	1.4.1
tibble	Simple Data Frames	3.1.8
tidyr	Tidy Messy Data	1.2.0
tidyverse	Easily Install and Load the 'Tidyverse'	1.3.2
utils	The R Utils Package	4.2.1