Title

The Balancing Act between Soil Quality and the Need for Guacamole: How Agricultural Practices Influence Soil Health

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The Balancing Act between Soil Quality and the Need for Guacamole: How Agricultural Practices Influence Soil Health

By

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DISSERTATION

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Dedication

To my husband – for keeping me calm when I would get stressed and being my pillar of support throughout this process and life.
To my parents and siblings – without your encouragement to pursue my dreams and never give up, I would not be here today.

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Abstract

Agricultural soils are net sources of atmospheric carbon globally. However, they have the potential to be carbon sequesters if managed using climate-smart agriculture (CSA) management practices that can improve global soil organic carbon (SOC) stocks. These practices are necessary to limit climate change and safeguard soil productivity and global food security. Soil also incorporates a wide range of environments that can comprise diverse microbial communities. Microbes serve important roles in decomposition of C and mineralization of N and are regulated by a variety of abiotic and biotic factors that can be influenced by different fertilizers, such as mineral and organic fertilizers. Additionally, soils serve as a primary sink and source of trace metals, especially in agricultural settings. Agricultural practices can lead to substantial heavy metal addition to soils through the use of fertilizers and pesticides. Whether soils serve as sinks or sources of trace metals depends on a wide range of factors that dictate their retention capacities for these metals.

The first chapter of this dissertation explores the effects of tree crop agriculture on soil carbon sequestration, with an emphasis on citrus and avocado groves in Southern California. It was found that tree crop agriculture may be a viable means of balancing both food production and carbon sequestration. The second chapter examines how mineral versus organic fertilizer treatments compare regarding their effect on nitrogen cycling, nitrogen uptake, and microbial communities in avocado and citrus groves. Contrary to expectations, there were no substantial differences observed within the target variables, although this outcome may be due to not much time having passed after fertilization treatments. The third chapter investigated the effect of long-term mineral versus organic fertilizer application on trace metal concentrations in soil

through sequential extractions. Both the DTPA and BCR extraction results suggest that organic fertilizer treatments increase bioavailability of Cu. Zn bioavailability was also found to increase with organic fertilizer per the DTPA extraction results and the XRF data, and Cr bioavailability was found to increase with organic fertilizer per the BCR extraction results.

Overall, this dissertation demonstrates the potential of tree crop agriculture for soil carbon sequestration and sets the foundation for future research comparing mineral and organic fertilizers on soil properties, with specific insights on how these fertilizers differ regarding trace metal bioavailability.

Introduction

Several agricultural soils are CO_2 sources because of soil disturbance and heavy cropping, emitting 5–6 Gt CO_2-eq yr^{-1} (Kantola et al., 2017). In 2020, agriculture contributed 11.2% of total gas emissions in the United States (USDA 2022; Figure 2). While agricultural soils are net sources of atmospheric carbon globally, if managed using climate-smart agriculture (CSA) management practices they also have the potential to be carbon sequesters (Bai et al., 2019). Some of the most effective CSA techniques include applying biochar amendments to soils, adding cover crops, and minimizing soil tillage (Bai et al., 2019). Enhanced weathering is another means by which carbon can be sequestered in agricultural soils, where crushed silicate rock is added as a soil amendment (Kantola et al., 2017). Finally, adjusting agricultural methods based on the regional climate can help minimize soil carbon losses (Sun et al., 2020). Efforts to improve global soil organic carbon (SOC) stocks are required to sequester atmospheric carbon to limit climate change in order to safeguard soil productivity and global food security (Bhattacharya et al., 2010).

The long-term balance among the additions of organic carbon from various sources and its losses through various pathways modify SOC stock in a specific area (Bhattacharya et al., 2010). For a long time, organic carbon content has been identified as a crucial component of soil quality, and therefore, conservation of SOC in cropland soils is a chief factor of the productivity and long-term stability of agricultural systems (Huang et al., 2010). Changes in carbon soil pools are usually linked to changes across carbon fluxes in terrestrial ecosystems (Chen et al., 2015). The precise method in which nitrogen regulates the carbon-cycle is still mainly unclear, which is a significant issue in model projections of future climates and ecosystems (Lu et al., 2011). Fruit tree ecosystems have the potential to remove C at a rate similar to those of forests; however, the C sink function of fruit tree ecosystems and the regulating ecosystem services have received relatively little attention (Montanaro, 2017).

Soil structure maintenance, including the formation of aggregates, is an important component of soil health and affects its ability to support food production and provide other ecosystem services (Kibblewhite et al., 2008). Soil aggregates are stable, amalgamated structures that are classified into different size classes (Bucka et al., 2019). Soil aggregation and structure is a significant characteristic of soil fertility by influencing root growth, spreading, and uptake of water and nutrients, while aggregates simultaneously aid in resistance to erosion (Huang et al., 2010). Soil organic matter (SOM) serves as a binding agent for aggregates, which are organized hierarchically (Six et al., 2004). SOC is closely linked with a wide array of physical, chemical, and biological properties of soil, thereby playing a key role in soil processes and functioning (Huang et al., 2010). Conventional agricultural practices, such as tilling, destroys soil aggregates (Tebrügge, 2003).

Micronutrients are elements that plants need in smaller quantities for growth in contrast to primary macronutrients like nitrogen, phosphorus, sulfur, and potassium (Hänsch & Mendel, 2009). Plants indicate diverse requirements for particular micronutrients; however, there are elements that are commonly acknowledged to be essential for all higher order plants which include chloride (Cl), boron (B), iron (Fe), copper (Cu), nickel (Ni), manganese (Mn), molybdenum (Mo), and zinc (Zn) (Hänsch & Mendel, 2009). Micronutrients serve obligatory roles in the biosynthesis of proteins, nucleic acids, growth materials, gene expression, metabolism of carbohydrates and lipids, stress tolerance, etc. via involvement with various physiologically active molecules and several enzymes (Aftab & Hakeem, 2020). With the use of traditional mineral fertilizers (NPK) and little/no use of organic fertilizer, widespread micronutrient deficiencies are occurring (Behera et al., 2019).

Zinc has emerged as the most widespread micronutrient deficiency in soils and crops worldwide (Das & Green, 2016). Zinc deficiency is a global public health problem, leaving ~2 billion people at risk for deficiency of this trace metal (Bafaro et al., 2017). Zinc deficiencies are common in many subtropical areas where avocados are grown (Crowley et al., 1996).

The productivity of many ecosystems is regulated by nitrogen availability (Vitousek et al., 2002). Nitrogen can be lost as particulate matter through the burning of biomass, volatilization into a gas, and nitrate leaching (Davidson et al., 2007). The production of reactive N through anthropogenic activities such as N fertilization, agricultural N_2 fixation, and combustion of fossil fuels has comprehensive consequences on productivity, ecosystem functions, biodiversity, and climate change (Hietz et al., 2010).

Soil microbial communities are regulated by abiotic and biotic factors such as soil moisture, soil texture, soil temperature, seasonality, and vegetation type (Mellado-Vasquez et al., 2019). Various communities of microbes differ in their capacity to adapt to differing soil nutrient levels, and under recurring fertilizer applications, microbial growth and activity can be altered due to physical, chemical, and biological changes (Wu et al., 2021). Research has long focused on the effect of agricultural management on biodiversity of higher organisms, and assessing microbial diversity has only recently become more accurate in the light of high-resolution sequencing (Hartmann et al., 2015). Sustainable agriculture is characterized by fungal dominance in soil microbial communities and a higher microbial metabolic efficiency compared to conventional agriculture (Chavarria et al., 2018).

Ideally, agricultural practices will prioritize both food production and climate change mitigation. Avocados are a major California crop and recommendations for growers are vital given a growing need to adapt sustainable agricultural practices particularly with intensifying climate change effects. Over the course of the next three chapters, we will address some concerns with cultivating avocados, citrus, tomatoes, and corn in California.

Chapter 1: Long-term impacts of agriculture on carbon, nitrogen, and trace metal storage

Abstract

Agricultural soils are net sources of atmospheric carbon globally. However, they have the potential to be carbon sequesters if managed using climate-smart agriculture (CSA) management practices that can improve global soil organic carbon (SOC) stocks. These practices are necessary to limit climate change and safeguard soil productivity and global food security. Soil plants and microorganisms take in atmospheric carbon and store it as SOC, which is contained within soil aggregates. Soil aggregates are stable structures that can be assigned to different size classes. POM is more sensitive to and has quicker response times to management practices and can be used as an indicator of turnover changes in soil organic matter (SOM). Therefore, carbon and nitrogen are more stable in microaggregates than macroaggregates. High yield agricultural practices, which are traditionally used in row crop ecosystems that do not have a high rate of CO_2 removal, have been concerned with adding only macronutrients without any regard for micronutrients. However, fruit tree ecosystems have the potential to remove CO_2 at a rate similar to those of forests. Therefore, avocados and citrus, the two of the most valuable tree crops grown in CA, might have a very different impact on soils than row crop ecosystems. This study examined this through the lens of long-term agriculture of avocado and citrus in native Southern California soils. While it was found that basic soil composition was unaltered by the introduction of tree crops, soils supporting tree crops had higher concentrations of both carbon and nitrogen. Moreover, nutrient concentrations also differed between grove and reference soil samples to

varying degrees. Ultimately, the findings of this study demonstrate that tree crop agriculture may be a viable means of balancing both food production and carbon sequestration.

Introduction

Avocados and oranges are considered to be two of the six most valuable crops for CA (Lobell et al., 2006). Avocados are native to Central America and the West Indies and 1856 was the first recorded planting of an avocado tree in California (CA) (California Department of Food and Agriculture, n.d.). Avocados are sensitive to frost and are primarily grown along the southern coast (California Department of Food and Agriculture, n.d.). San Diego leads CA in the production of avocados, accounting for 47% of CA avocado market value (EPA, 2015). The introduction of citrus fruits to CA dates to 1769 and the first planted orchard was recorded in 1804 (California Department of Food and Agriculture, 2016). Commercial citrus production in CA comprises 30% of citrus production and 42% of national value in the United States (California Department of Food and Agriculture, 2013).

Soil structure maintenance, including the formation of aggregates, is an important component of soil health and affects its ability to support traditional food production and provide other ecosystem services (Kibblewhite et al., 2008). Soil plants and microorganisms take in atmospheric carbon and store it as soil organic carbon (SOC) which is contained within soil aggregates. Soil organic carbon (SOC) is directly related to the formation and stability of aggregates (Huang et al., 2010; Tripathi et al., 2014).

Microaggregates form within macroaggregates and particulate organic matter (POM) from roots aids the formation of aggregates (Six et al., 2004). Therefore, POM could not be easily available for degradation due to being physically stabilized in aggregates and also composed of carbon that is biologically resistant (Zhou et al., 2010).

SOM consists of a range of pools that fluctuate in their physical and chemical characteristics, turnover rates, and decomposability (Liao et al., 2006). In order to better manage agricultural systems, a more comprehensive knowledge is needed to understand how nutrients, particularly carbon and nitrogen are stored in soil. SOM is mainly stabilized in stable microaggregates, while across soil types and perturbations, alterations in the turnover rate of macroaggregates can influence SOM stabilization (Six et al., 2004). POM can have a turnover time ranging from months to years and is composed of fine root fragments and organic debris (Zhou et al., 2010). POM is easily decomposable for soil microbes and also acts as a nutrient pool for plants short-term (Zhou et al., 2010). Older SOM is usually more resilient to decay and contained in microaggregates, which are frequently found within macroaggregates that are combined together by roots and fungal hyphae (Liao et al., 2006). POM is more sensitive to and has quicker response times to management practices and can be used as an indicator of turnover changes in SOM (Zhou et al., 2010). Therefore, carbon and nitrogen in microaggregates will be more stable than in macroaggregates.

An important component of organic matter dynamics is the formation of soil aggregates that aid in stabilizing soil structure, which is significant for aeration of soils and their resistance to erosion (Grunwald et al., 2016). Microaggregates display slower turnover rates and greater stability in comparison with macroaggregates, indicating that microaggregates are more valuable for long-term carbon sequestration (Huang et al., 2010).

Levels of SOC can be controlled via crop rotation and fertility maintenance, which is inclusive of inorganic fertilizers and organic manure, tillage methods, and other cropping system components (Bhattacharya et al., 2010). As the human population continues to grow, there will be a need for increased efficiency of agricultural systems and land use changes. Concurrently, present agricultural lands will need to be cultivated intensively and efficiently (Schlesinger & Andrews, 2000). Alterations in soil organic matter and nutrient pools following the conversion of native systems to agriculture have been well recognized (Compton & Boone, 2000). As climate change affects temperature, precipitation, and other agricultural related variables, it will have multifaceted impacts on global food security (Jia et al., 2019). This will lead to more land use change which in turn can exacerbate global climate change.

Food security is a growing concern globally, related to obtaining food, and the quality and variability of food (Tripathi et al., 2015). Traditionally, high yield agricultural practices have been concerned with adding only macronutrients without any regard for micronutrients (Imtiaz et al., 2010). Crops harvest both macro- and micronutrients from the soil, effectively mining the soil of its nutrients (Imtiaz et al., 2010; Jones et al., 2013).

Micronutrients are engaged in almost all metabolic and cellular activities, such as gene regulation, hormone perception, etc. (Hänsch & Mendel, 2009). Many vital metal ions are redox-active which serves as a source for their existence as catalytically active cofactors in metalloenzymes (Hänsch & Mendel, 2009). A shortage of micronutrients leads to significant constraints on productivity, stability, and sustainability of soil (Aftab &

Hakeem, 2020). Zinc is of particular interest in avocado cultivation because it is often deficient in many subtropical areas where avocados are grown, leading to issues in fruit quality and tree health (Crowley et al., 1996).

Many studies show row crops contribute to depletion of micronutrients and loss of soil carbon, especially in arid or semi-arid regions (Smith et al., 2008; Imtiaz et al., 2010; Jones et al., 2013). However, fruit tree ecosystems have the potential to remove CO_2 at a rate similar to those of forests (Montanaro, 2017). Therefore, avocados and citrus, the crops analyzed in this study, might have a very different impact on soils. The main hypotheses of this research are that grove soils could have a net positive effect on the formation of larger macroaggregates, C and N storage, and micronutrient concentrations.

Methods

Field site and sampling

The study site for this research project is the avocado and citrus grove that is situated on Santa Margarita Ecological Reserve (SMER). SMER was established in 1962, is managed by San Diego State University (SDSU), and comprises land owned by SDSU, the SDSU Research Foundation, the U.S. Bureau of Land Management, the California Department of Fish and Wildlife, and the Nature Conservancy. The grove was acquired by SDSU circa 1996, and for the first 20 years it was managed by an agricultural company that administered fertilization treatments and harvested the crops. Since 2014, the grove has been left largely undisturbed by humans, apart from being watered and harvested. The grove comprises 20-acres and lies approximately between

117°11’47.09” W and 117°11’17.68” W, and 33°26’03.68” N and 33°25’55.88” N. The region has a 30-year (1981-2010) rainfall average of 358 mm/yr.

Figure 1. Map of field site with zones and sample points indicated. Yellow pins indicate grove samples, teal pins indicate reference samples.

Three zones were chosen based on elevation and drainage patterns on Google Earth. Each zone was subdivided into two subzones that had comparable elevation and drainage patterns. Six reference points were also identified for comparison with grove soils (Figure 1).

From each subzone, six soil collection points were randomly chosen. If a collection point was on a tree or right by the base of a tree, the actual collection point was moved three feet away from the tree. Soil samples were collected from all the subzones over a period of three weeks in October of 2019. An additional six reference samples were collected from surrounding native soils over a period of two weeks in

January 2021. The soil samples were collected with the use of a 15cm trowel that was plunged into the soil and lifted out in order to keep the aggregates intact.

Soil fractionation (water stable aggregates)

The collected soil was weighed, airdried, and then weighed again. After the soil was airdried, approximately 100g from each sample was weighed and sieved in a 19-liter bucket. Exact weights were recorded per sample. The sieve sizes used were 2000, 500, and 250 microns, which were stacked from largest to smallest size. These sizes were chosen to look at larger macroaggregate stability. The bucket was filled with water up to half the topmost sieve in order to not let the soil get washed over the side. This was then set on a rotator at 75RPM for 5 minutes. Each soil fraction was separately dried in a drying oven set at 100°C. After drying, the soil was weighed again.

The oven-dried soil fractions as well as the original bulk soil were used for the carbon and nitrogen analysis. Approximately, 20-40mg were weighed in Costech 5x9mm tin capsules. Exact weights were recorded per sample. The capsules were then pressed into compact cubes and stored for mass spectrometry analysis. The prepared tin capsules were run on a Costech Analytical Elemental Combustion System coupled with a Thermo-Finnegan Mass Spectrometer.

Diethylenetriaminepentaacetic acid (DTPA) & HNO_3 extraction

DTPA extractions were done according to Lindsay and Norvell (1978) and HNO_3 extractions were done according to Groenenberg et al. (2017). The extractions were analyzed using PerkinElmer Inductively Coupled Plasma (ICP-OES). DTPA extractions were conducted to ascertain the availability of trace metals to plants, while HNO_3 extractions were conducted to determine how metals are occluded in particles. Both these methods were utilized because organic matter can either bind metals and make them unavailable or chelate them (Fan et al., 2016) which makes them easier to be accessed by plants (Zhang et al., 2016).

Soil texture analysis, POM & organic matter

Texture and particle size analysis was done by standard methods laid out by Smith and Mullins (1991), and the sieve sizes used were 2000, 500, 250, and 63 microns. POM was obtained by using the density separation method (Strickland & Sollins, 1987). After density separation from the mineral soil, the light organic fraction was captured on a filter, rinsed, dried, and weighed. Organic matter content was evaluated through combustion (Salehi et al., 2011).

Statistical analysis

Statistical analyses were conducted with R-Studio and Microsoft Excel. ANOVAS and pairwise t-tests were used to determine significance. Statistical analyses were conducted on log-converted values to ensure equal variance.

Results

There were no significant differences between grove and reference soil samples regarding average percent POM, average percent organic matter, percent gravel, percent sand (coarse, medium, fine), percent silt, and percent clay (Table 1). There was a significant difference regarding pH (Table 1). The masses of texture analysis fractions were similar across both reference and grove soil samples for all soil fractionation sizes (Table 1).

Table 1. Basic soil composition measurements for reference and grove soil samples. Asterisk indicates a significant difference.

	R	Grove
pH*	6.39	7.50
Average %POM	2.07	0.82
%gravel	11.30	9.86
%coarse_sand	18.29	22.09
%medium_sand	16.46	18.84
%fine_sand	24.86	21.55
%silt	23.18	24.74
%clay	5.92	2.92

The weighted nitrogen concentration was significantly higher in soil samples from the grove than in samples taken from the reference site, but only in the soil fractionation containing particles with diameters greater than 2000 μm (Figure 2a). In all other fractionation sizes, there was no difference in weighted nitrogen concentration between reference samples and grove samples.

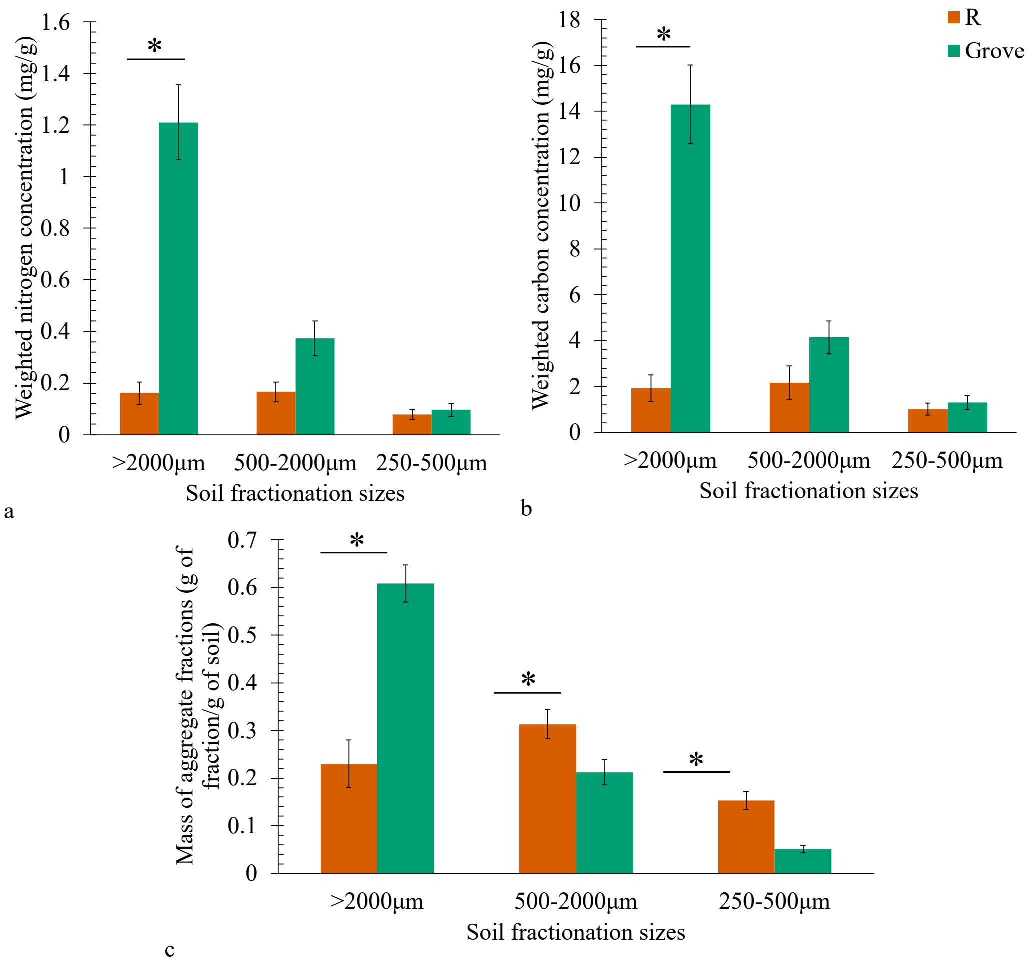


Figure 2. a) Weighted concentration of nitrogen in different soil fractionation classes between grove and reference samples. b) Weighted concentration of carbon in different soil fractionation classes between grove and reference samples. c) Mass of aggregates in different soil fractionation classes between grove and reference samples. Asterisks indicate significant differences ($p<0.05$).

Similar to nitrogen, the weighted carbon concentration was also significantly higher in soil samples from the grove than in samples taken from the reference site in the soil fractionation containing particles with diameters greater than 2000 µm (Figure 2b). Also similar to nitrogen, in all other fractionation sizes, there was no difference in weighted carbon concentration between reference samples and grove samples.

The mass of aggregate fractions was significantly higher in grove soil samples than in reference samples within the >2000 μm soil fractionation (Figure 2c). However, the inverse was true in the other soil fractionation sizes; for the 250-500 μm and 500-2000 μm fractionations, the mass of aggregate fractions was higher in the reference soil samples than in those from the grove.

Nitrogen and carbon concentrations were higher for both the fractionated (sumNagg and sumCagg) and unfractionated (Bulk_N and Bulk_C) grove soil samples than they were in the reference samples (Figure 3 a & b).

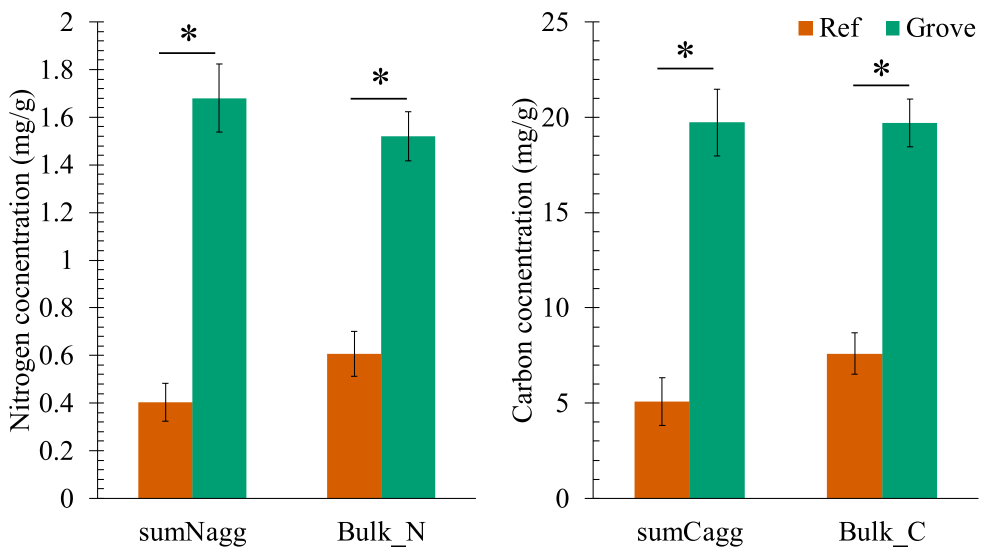


Figure 3. a) Nitrogen concentrations in the sum of soil fractionation classes (sumNagg) and total unfractionated soil (Bulk_N). b) Carbon concentrations in the sum of soil fractionation classes (sumCagg) and total unfractionated soil (Bulk_C). Asterisks indicate significant differences ($p<0.05$).

DPTA extractions indicated differences in concentrations of Mn, Fe, and Zn between grove and reference soil samples (Figure 4). Mn and Zn concentrations were higher in grove soil samples, while Fe concentration was greater in the reference soil samples. There was no difference between grove and reference soil samples with regards to Cu concentration.

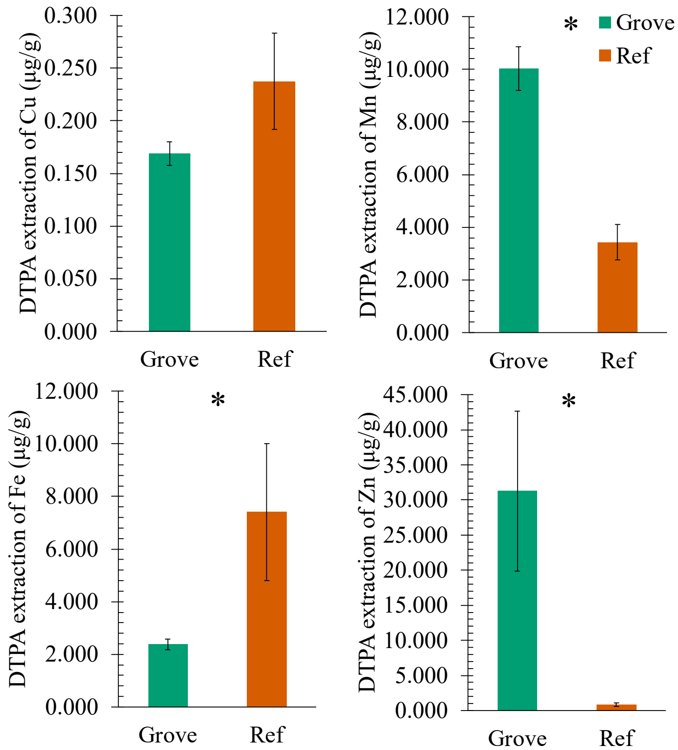


Figure 4. DTPA extractions of Cu, Mn, Fe, and Zn in grove and reference soils. Asterisks indicate significant difference ($p<0.05$).

The average concentrations of acid-extractable elements in non-combusted soil were higher in grove soil samples with regards to K, Mg, P, Zn, and Mn than in reference soil samples (Figure 5). However, there was no difference in average concentrations in acid-extractable elements between grove and reference soil samples with regards to Ca, Fe, and Na.

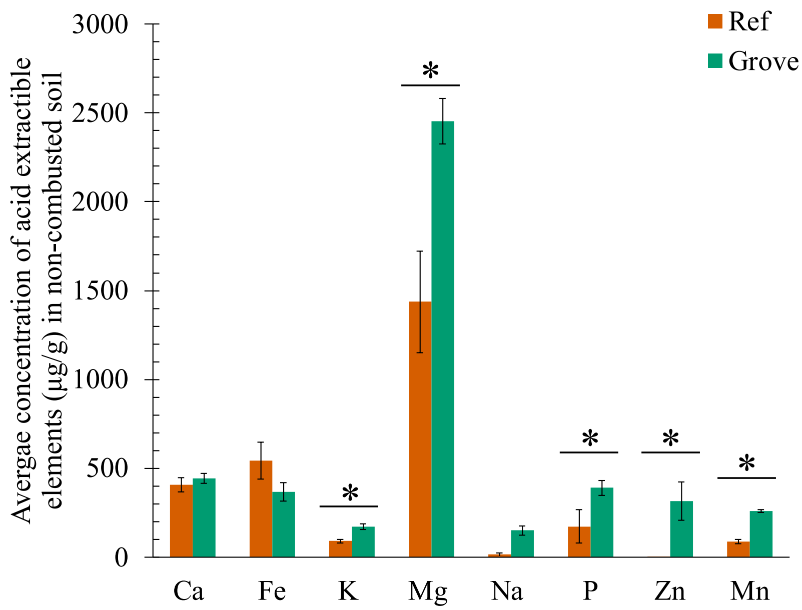


Figure 5. Comparison of grove and reference soil samples regarding average concentration of acid-extractable elements in non-combusted soil. Asterisks indicate significant difference (p<0.05).

The difference in the concentration of Fe between combusted and non-combusted soils was greater in reference soil samples than in grove soil samples (Figure 6). However, the converse was true of Mn, with the difference in concentrations between combusted and non-combusted soils being greater in grove soil samples. The difference in the concentration of P between combusted and non-combusted soils was similar in both grove and reference soil samples.

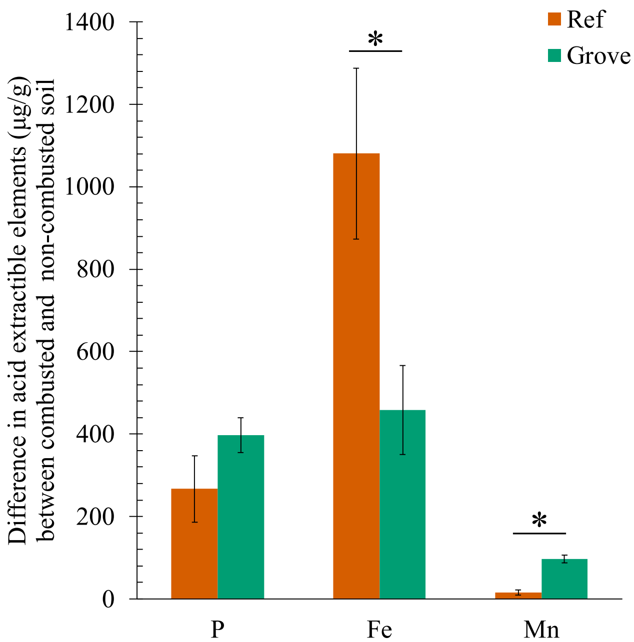


Figure 6. Comparison of grove and reference soil samples regarding organic matter-associated elements. Asterisks indicate significant difference (p<0.05).

Discussion

Given that there was no difference in basic soil composition between grove and reference soils (Table 1), it is safe to assume that any differences between the two soil types regarding nutrient composition can be attributed to the introduction of non-native vegetation to the grove. The grove did not have any fertilizer amendments in five years prior to soil sample collection. Leaf litter was a source of organic matter and soil cover for grove soils. Nitrogen and carbon concentrations were higher in grove soil samples than in reference soil samples for the >2000µm soil fraction (Figures 2 a & b). Additionally, the mass of aggregate fractionations was greater in grove soil samples for the >2000µm soil fraction but lesser in grove soil samples for the smaller fraction sizes (Figure 2c). The mineral associated organic matter (MAOM) likely had little influence over the C and N storage due to no significant difference found between the bulk soils and the aggregates (Figure 3).

The overall higher concentrations of carbon and nitrogen in grove soils (Figures 2 & 3) are contrary to traditional agriculture which breaks up aggregates through tillage (Zheng et al., 2018). Macroaggregates are especially susceptible to being broken down as they are composed of organic-binding agents that are more rapidly degradable than those found in microaggregates (Scow, 1997). Due to less disruption, native soils would tend to have better macroaggregate stability and consequently higher carbon and nitrogen concentrations in the larger aggregates. It is possible that there may not have been as much tilling involved when fertilizer amendments were regularly added until 2014, resulting in the preservation of the larger aggregates in the grove soils. Also, the supply of organic matter in the form of leaf litter likely contributed to better aggregate formation and stability in grove soils, as fresh litter substrates contribute to macroaggregate formation (Howe & Smith, 2021).

It should be noted that the fertilizer treatments administered to the grove soils until 2014 only added NPK, so they would not have directly impacted bioavailability concentrations of Mn, Zn, Fe, and Cu. It is likely that Cu and Fe concentrations are naturally high in the regional soil's parent materials, which would explain why their bioavailability concentrations were relatively high in the reference soils (Figure 4). Avocado and citrus trees take up Fe (Pestana et al., 2011; Salazar-García, 1999) which could explain why Fe concentrations were significantly lower in the grove soil samples. Although both avocado and citrus also take up Cu (Mattos-Jr et al., 2023), Cu is also associated with disease in avocado crops (Ramírez-Gil et al., 2021), which could explain a reduced uptake and why there was no difference among grove and soil samples regarding Cu concentrations. Additionally, Cu concentrations in avocados have been documented to be less than Fe concentrations (Reddy et al., 2014). While micronutrients like N, P, and K are commonly reported as deficient in soils globally, micronutrients including Zn, Fe, Cu, and Mn are also often found deficient (Shukla et al., 2021). The higher concentrations of Mn and Zn in grove soil samples could be an indication that the avocado and citrus trees are mining the soil for these micronutrients (Buckeridge et al., 2022), bringing them to the upper layers of the soil where they would not normally be present. Additionally, leaf litter inputs could be returning them to the upper layers of the soil.

The differences observed between grove and reference soil samples with regard to nutrient concentrations in the acid-extractable results (Figure 5) provides an indication of what elements have greater bioavailability but not any information regarding the concentrations of these elements occluded in oxides. The higher bioavailability concentrations of P and K in grove soil samples could be due to the previous NPK fertilization amendments. The higher bioavailability concentrations of Mg, Mn, and Zn in grove soils can likely be explained by avocado and citrus plants mining for these micronutrients and leaf litter resupply (Buckeridge et al., 2022). The relatively high concentrations of Mg in both the grove and reference soil samples suggests that in addition to being mined by the grove trees, Mg is also likely supplied by the soil's regional parent materials.

The results shown in Figure 6 give an indication of the relative concentrations of elements occluded in oxides. The difference between grove and reference soil samples regarding Fe concentrations is likely due to Fe being present in the soil's regional parent materials, with some portion of it being converted into a form that can be absorbed by the citrus and avocado trees, resulting in a lower concentration in the grove soils as avocado and citrus trees take up Fe (Pestana et al., 2011; Salazar-García, 1999). The higher concentration of Mn in grove soils is likely due to the citrus and avocado trees mining for Mn, with a portion of the mined Mn being occluded in oxides with the addition of organic matter supplied by leaf litter. The similar levels of P in the grove and reference soils suggest that in the five years between the last application of fertilizer and the collection of samples, the avocado and citrus trees had already withdrawn the added P.

As the effects of climate change continue to be exacerbated by exponentially increasing levels of greenhouse gases, there is a pressing need for carbon sequestration. One significant avenue in this endeavor is the conversion of agricultural soils from atmospheric carbon sources to carbon sinks. Ideally, agricultural practices will prioritize both food production and climate change mitigation. However, this can only be made possible with a comprehensive understanding of a soil's composition and how it can be affected by the plants it sustains. This study examined this through the lens of long-term agriculture of tree crops, specifically avocado and citrus, in native Southern California soils. While it was found that basic soil composition was unaltered by the introduction of tree crops, soils supporting tree crops had higher concentrations of both carbon and nitrogen. Moreover, nutrient concentrations also differed between grove and reference soil samples to varying degrees. Ultimately, the findings of this study demonstrate that tree crop agriculture may be a viable means of balancing both food production and carbon sequestration.

Chapter 2: Short-term impacts of mineral versus organic fertilizer treatments on nitrogen cycling and microbial communities

Abstract

Organic fertilization is used as an alternative to mineral N fertilization and has many advantages including reducing the need for chemical N application and enhancing nitrogen use efficiency. The consequences of replacing mineral fertilizer with organic fertilizer can vary because of complex interactions with the organic fertilizer substitution rate, overall nutrient supply, and the cropping system utilized. Organic fertilization fosters the accumulation of soil N reserves, which signifies the ecosystem has the capacity to sequester N. The use of organic fertilizers has also led to a decrease of the global warming potential in some agricultural systems. Additionally, microbes serve important roles in decomposition of C and mineralization of N and are regulated by a variety of abiotic and biotic factors that can be influenced by different fertilization approaches. The objective of this study was to research how mineral versus organic fertilizer treatments compared regarding their effect on nitrogen cycling, nitrogen uptake, and microbial communities in avocado and citrus groves. Contrary to previous studies comparing organic and mineral fertilizer treatments, there were no substantial differences observed within the target variables, although this could be due to not much time having passed after fertilization treatments. This study sets a foundation for future research and serves as a baseline for short term fertilization results.

Introduction

Nitrogen (N) is an essential element in nutrition due to its elevated requirement by plants for the formation of proteins, nucleic acids, vitamins, hormones, chlorophyll pigments, and other organic compounds; essentially it is physically incorporated in most catalytic molecules (Fikry et al., 2020). Additionally, nitrogen influences the absorption and allocation of all other elements and is specifically important to trees during flowering and fruiting (Fikry et al., 2020). Humanity uses approximately 83 million tons of N annually and 50% of the global population relies on nitrogen fertilization for food production; of those using N fertilizer, 60% employ it for major cereal crops such as rice, wheat, and maize (Alkhaldi et al., 2012). Many ecosystems worldwide are still limited by Ndespite adaptations to utilize this nutrient efficiently (Galloway et al., 2004). The limitation of productivity due to N, although natural, makes supporting agriculture to feed a growing human population unsustainable. Thus, anthropogenic activity results in substantial alterations of the N cycle in air, land, and water (Galloway et al., 2004).

The global manufacturing of reactive N has approximately doubled compared to preindustrial times(Galloway et al., 2004) and because reactive N does not persist in the atmosphere for long periods, variation in local deposition depends on regional industrial and agricultural activities (Hietz et al., 2010). N use efficiency is low because merely 50% of fertilizer N applied is taken in by crops, 2-5% is retained in the soil, and the remaining 45-48% leaches into groundwater or is discharged into the atmosphere (Toselli et al., 2019). Agricultural N leaching accounts for approximately 80% of the 1.2 million tons of N that discharges into the Gulf of Mexico leading to hypoxia (Wolz et al., 2018). Continued application of chemical fertilizer leads to imbalances of soil nutrients and contributes to soil acidification and salt accumulation, resulting in the loss of N (Wu et al., 2021). Conversely, constant cropping without utilization of fertilizers leads to decrease of soil nutrients and negatively alters biochemical parameters (Wu et al., 2021).

Organic fertilization is used as an alternative to mineral N fertilization and has many advantages: augmenting the availability of all nutrients, reducing soil salinity, enriching soil fertility, increasing water retention and soil organic matter, enhancing biological activity of microflora, natural hormones, and antibiotics, reducing the need for chemical N application (Fikry et al., 2020) and enhancing nitrogen use efficiency (Wei et al., 2020). The consequences of replacing mineral fertilizer with organic fertilizer can vary because of complex interactions with the organic fertilizer substitution rate, overall nutrient supply, and the particular cropping system utilized (Wei et al., 2020). Organic fertilization strategies foster the accumulation of soil N reserves which signifies the ecosystem has the capacity to sequester N (Toselli et al., 2019). When organic fertilizer is used, it leads to a decrease of the global warming potential by 116 kg CO_2 eq ha^{-1} in maize systems (Wei et al., 2020).

Babbar & Zak (1994) examined shaded and unshaded coffee agroecosystems and found that shaded coffee farms had higher N availability, but lower leaching rates than the unshaded farms, indicating that N losses are reduced under a multi-crop scenario. Utilizing ^{15}N labeled fertilizers, Hamid & Sarwar (1976) found that ammonium nitrate was utilized more than urea in wheat crops when fertilizer was added in a six split application strategy. Patnaik (1965) studied isotopically marked ammonium sulfate to differentiate between soil and fertilizer derived N and traced the transference of fertilizer N within rice crops, finding that only 30-40% of applied fertilizer is retrieved in the plant.

In citrus and avocado crops, N management is dependent upon different seasons for growth and development; in California from April to November there is rapid uptake of N but from November to March there is no net uptake of N (Khalsa et al., 2018). Requirement for N is low from December to February because of remobilization from shoots to fruit which fulfills fruit N demand (Khalsa et al., 2018). As the trees become established, the quantity of N allotted to vegetation reduces and for avocado yield, the timing of N appears to be more impactful than the overall N rate (Khalsa et al., 2018).

Soil incorporates a wide range of environments that can comprise diverse microbial communities (Fierer, 2017). Microbes serve important roles in decomposition of C and mineralization of N (Wu et al., 2021). The predominant rate determinant of microbial processes is soil temperature (Lal 2004; Oertal et al., 2016; Tang et al., 2018). The biogeochemistry of N is predominantly reliant on redox reactions facilitated by microorganisms, and to a reduced degree on long-term recycling via the geosphere (Canfield et al., 2010). Over the course of evolution, a limited number of microbes evolved the capability of converting N_2 to reactive nitrogen such as NH_3, NH_4, etc. (Galloway et al., 2004). Microbes introduce nitrogen into the soil to meet growth requirements at high carbon to nitrogen ratios; while at low carbon to nitrogen ratios, nitrogen is emitted into the ecosystem because it is in surplus for growth requirements (Chapin et al., 2002). In the previous chapter it was found that basic soil composition was unaltered by the introduction of tree crops, and that soils supporting tree crops had higher concentrations of both carbon and nitrogen. The investigation of microbial community composition could provide a novel glimpse into soil health of a managed ecosystem.

The tracer technique has been used extensively in the investigation of nutritional problems of crops (Nömmik, 1966). While much research has been done on more traditional crops, there is scarce data examining how mineral versus organic fertilizer affects avocado trees and examining how different forms of ^{15}N cycle through avocado soil. The objective of this study was to research how mineral versus organic fertilizer treatments compared in regard to their effect on nitrogen cycling, nitrogen uptake, and microbial communities.

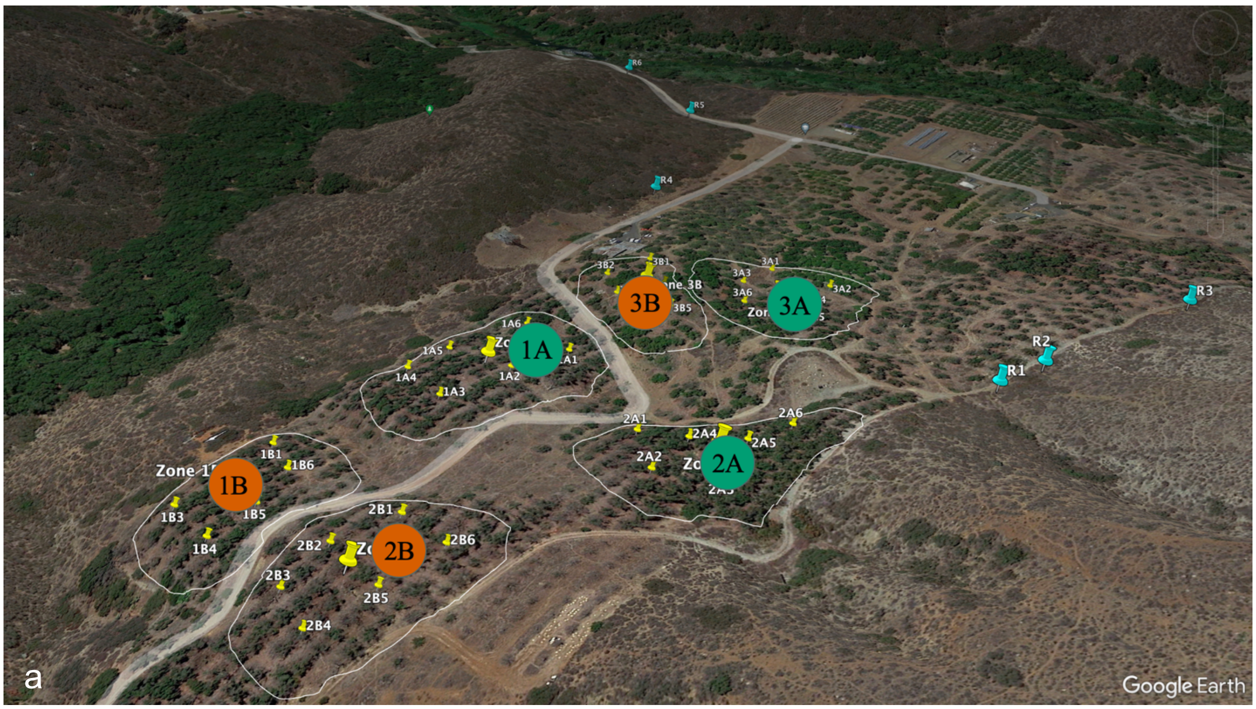
Methods

Field site

The study site for this research project is the avocado and citrus grove that is situated on Santa Margarita Ecological Reserve (SMER). SMER was established in 1962, is managed by San Diego State University (SDSU), and comprises land owned by SDSU, the SDSU Research Foundation, the U.S. Bureau of Land Management, the California Department of Fish and Wildlife, and the Nature Conservancy. The grove was acquired by SDSU circa 1996, and for the first 20 years it was managed by an agricultural company that administered fertilization treatments and harvested the crops. Since 2014, the grove has been left largely undisturbed by humans, apart from being watered and harvested. The grove comprises 20-acres and lies approximately between 117°11’47.09” W and 117°11’17.68” W, and 33°26’03.68” N and 33°25’55.88” N. The region has a 30-year (1981-2010) rainfall average of 358 mm/yr.

Fertilizer treatment

The grove was divided into six sections. Half the sections received mineral fertilizer (B zones) and the other half received organic fertilizer (A zones) (Figure 1a). Ten trees were randomly selected in each section and were labeled by nailing a tag onto the bark. From the pre-labeled trees, two focal trees from each section (12 plots



total) were selected for the experiments described below. The mineral fertilizer used was YaraMila 15-15-15 and the organic fertilizer used was BioFlora Crumbles. The fertilizer treatments were added to the soil on October 22, 2021.

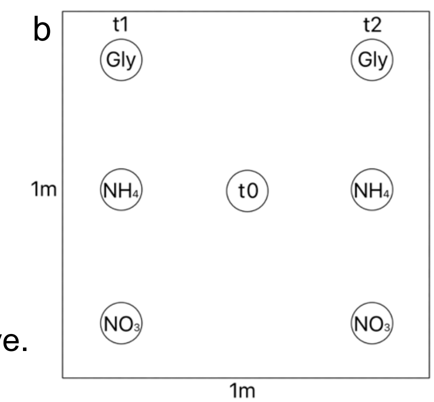


Figure 1. a) Map of SMER grove. b) Nitrogen label experiment setup.

Nitrogen labels

Ten days after fertilization, 1m x 1m plots were established within the dripline of each focal tree (Figure 1b). Soil cores were constructed using PVC pipe, with a length of 15cm and a diameter of 3.75cm. Resin bags were constructed from nylon stocking material and 10g of mixed bed ion exchange resin (Dowex® Marathon™ MR-3 hydrogen and hydroxide form). Resin bags were inserted at the bottom of each core and held in place with plastic end caps, with holes drilled to allow drainage. The cores were then placed back into their original holes, except the t0 cores, which were used for background ^{15}N measurements.

The cores were injected with ^{15}N labels (glycine, NH_4, NO_3). The ^{15}N labels (98 atom%) were injected into soil cores using a 50cc syringe and a 2mm stainless steel tube, crimped at the end to avoid blockage with soil, with slits near the tip to allow egress of solution. ^{15}N solutions were injected evenly along the profile of the soil core by pulling out the syringe while depressing the plunger. Based on estimates of soil volumetric water content, a final soil pore water concentration of 1μM of ^{15}N label was added, at least one order of magnitude lower than typical soil concentrations so that the labeling treatment did not appreciably increase the total concentration of NH_4, NO_3 or glycine.

Soil cores were sampled at one month (t1) and five months (t2) after ^{15}N label injection. Soils and resins were extracted in 25ml KCl (2M) per 10g. NH_4 and NO_3 concentrations were measured with colorimetric methods adapted for plate reader (Lipson et al., 2010). Total N in extracts was analyzed as NO_3 after persulfate digestion (Hosomi & Sudo, 1986). ^{15}N isotopic ratios of NO_3, NH_4 and total N in extracts were measured by diffusion onto acidified GF/A glass filter disks, followed by stable isotope mass spectrometry using a Thermo Finnigan DeltaPlus (Stark & Hart, 1996).

Leaf sampling

Leaf sampling was done according to agricultural procedures laid out in Selladurai & Awachare (2020) on October 29, 2021. Mature leaves were collected from quadrants around each tree. The four quadrants around each tree were selected to correspond with the northern, southern, eastern, and western sides of the tree to account for sunny versus shady sides. Leaves were collected at a height of 185 cm.

Genomic sampling and sequencing and microbial community analyses

Ten soil samples (15 cm depth, 3.75 cm diameter) were collected from each plot in March 2022, and returned to the laboratory where subsamples were immediately frozen at -20C for DNA extraction (QIAGEN DNeasy PowerSoil Kit). DNA was quantified using both a nanodrop spectrophotometer and a qubit fluorometer. Shotgun metagenomes were sequenced with Illumina Hi-Seq (paired-end 300 bp, GeneWiz/Azenta). The Department of Energy Systems Biology Knowledgebase (KBase) was used to process the raw read libraries (Trimmomatic, FastQC) and produce Kaiju taxonomy & FAMA profiles of nitrogen-cycling genes (Arkin et al., 2018). The average total number of sequences was 39,672,404 for the five mineral soil samples and 40,165,077 for the five organic soil samples.

Statistical analysis

Statistical analyses were conducted with R-Studio. ANOVAs, Kruskal-Wallis tests, Relative abundance graphs and NMDS plots, and pairwise t-tests were used to determine significance. Statistical analyses were conducted on log-converted values when needed to ensure equal variance.

Results

Percent N was higher in leaf samples taken from trees growing in soils treated with mineral fertilizer in comparison to those from soils treated with organic fertilizer (Figure 2). Leaves from both soil types had percent N values above 2%.

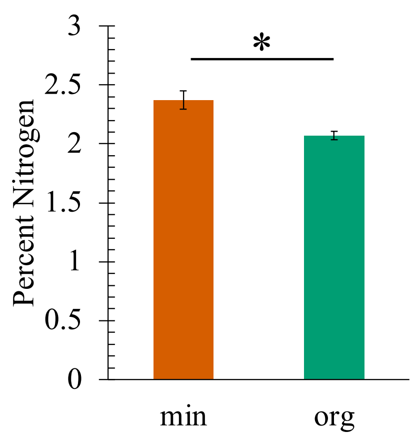


Figure 2. Percent nitrogen in leaves one week after fertilization between mineral and organic fertilizer treatments. Asterisk indicates p-value < 0.05.

The labeled glycine was different from labeled NH_4 and NO_3 across both t1 and t2 for $\delta^{15}N$ (Figure 3). $\delta^{15}N$ in t0 was also different from t1 and t2. There were no differences between mineral and organic treated soils regarding $\delta^{15}N$.

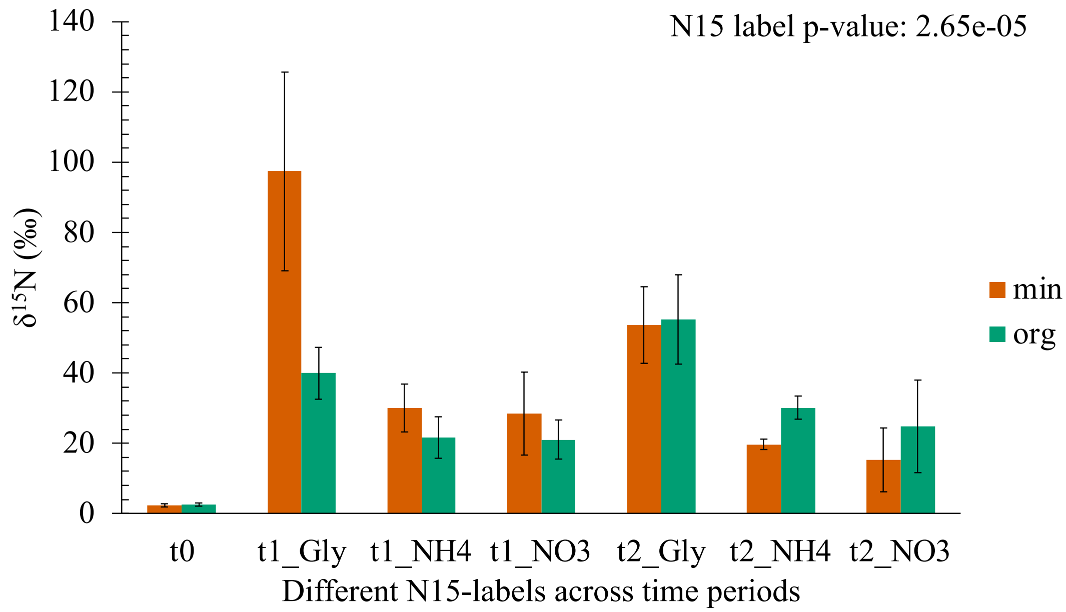


Figure 3. Bulk soil enriched with ^{15}N-Glycine and ^{15}N-NH_4 across three different time periods, mineral versus organic fertilizer treatment, and ^{15}N labels (Glycine, NH_4, NO_3). Significant difference was found between the ^{15}N labels with the t1 Glycine label being significantly different from other labels. Time period t0 was also significantly different from t1 and t2.

For extracted NO_3 and NH_4, δ^{15}N differed between soils and resins across all ^{15}N labels (Figure 4). However, there was no difference between soils treated with organic versus mineral fertilizers regarding δ^{15}N.

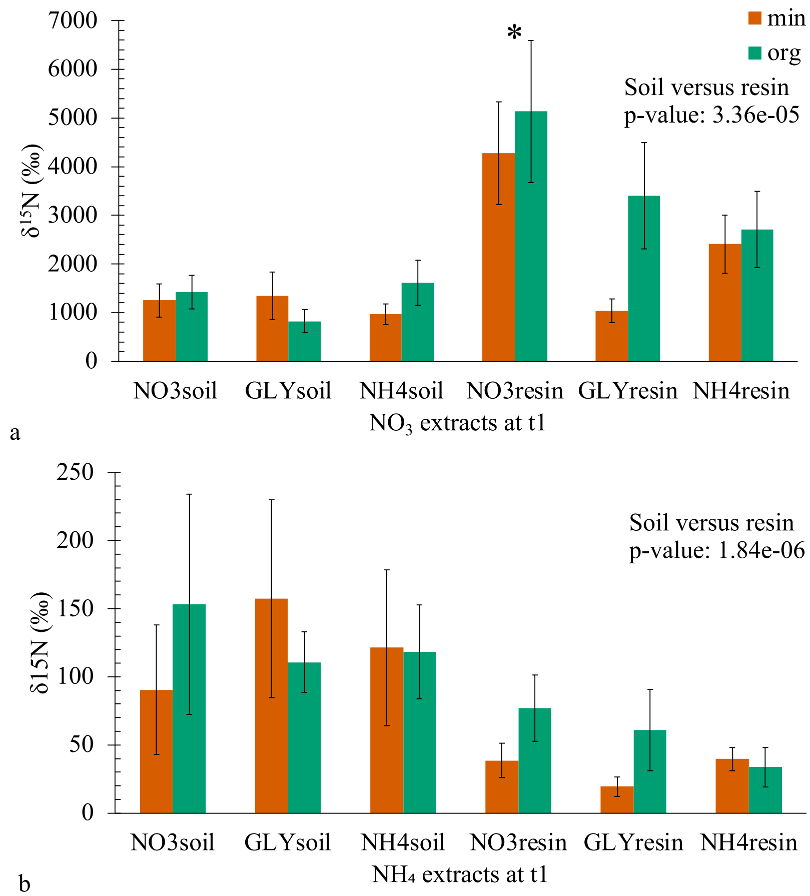


Figure 4. a) Relative difference in ^{15}N and ^{14}N for NO_3 extracts in time period t1, across mineral versus organic fertilizer treatment, and ^{15}N labels (Glycine, NH_4, NO_3). Significant difference was found between soil and resin. b) Relative difference in ^{15}N and ^{14}N for NH_4 extracts in time period t1, across mineral versus organic fertilizer treatment, and ^{15}N labels (Glycine, NH_4, NO_3). Significant difference was found between soil and resin.

There was no difference regarding mineralization rates between mineral and organic fertilizer treatments for t1 (p-value = 0.2002) and t2 (p-value = 0.8728) (Figure 5), nor was there a significant difference between mineral and organic treatments for either time period (t1 p-value = 0.2002; t2 p-value = 0.631) when looking at net nitrification (Figure 6). Additionally, there was no significant difference between mineral and organic treatments for either time period (t1 p-value = 0.2623; t2 p-value = 0.2002) when looking at potential leaching (Figure 7).

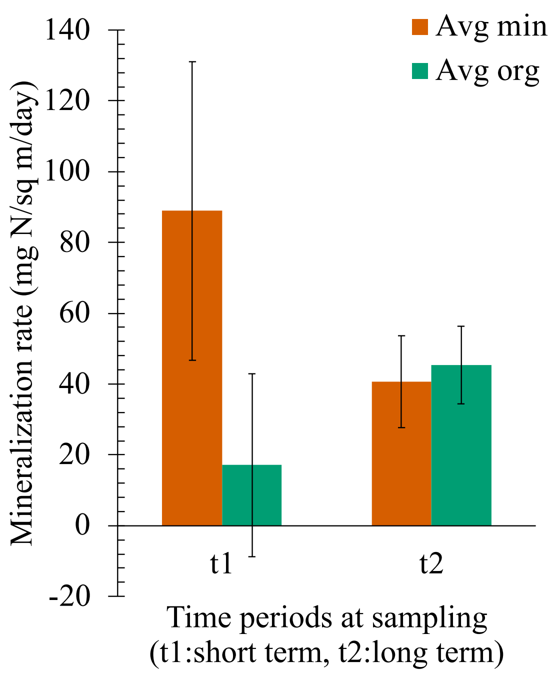


Figure 5. Average mineralization rates between mineral and organic fertilizer treatments for t1 and t2.

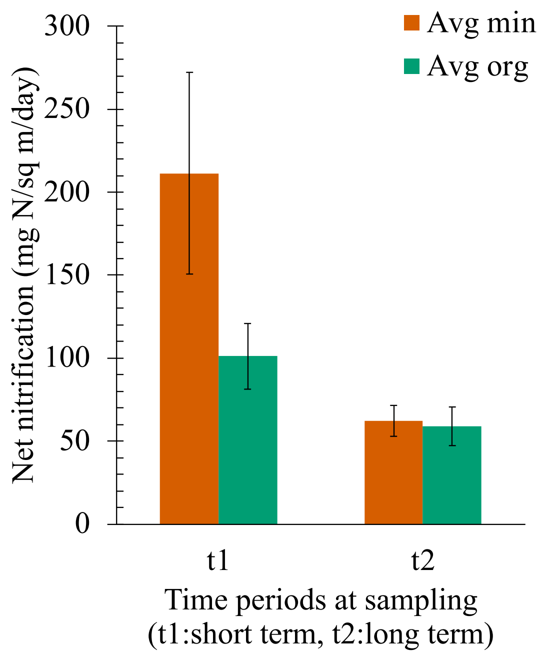


Figure 6. Average net nitrification rates between mineral and organic fertilizer treatment for t1 and t2.

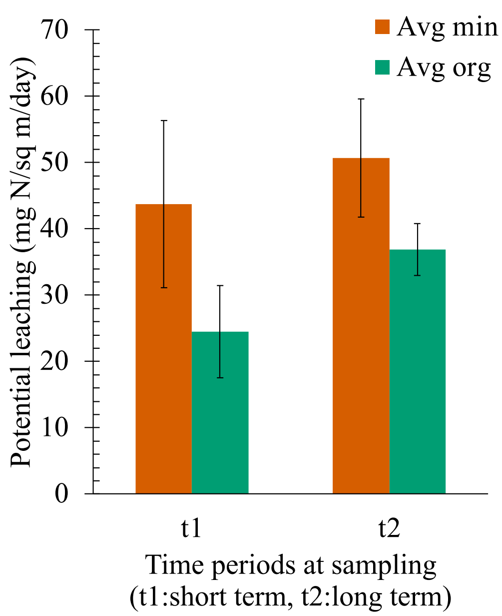


Figure 7. Average potential leaching rates for mineral and organic fertilizer treatment between t1 and t2. There was no significant difference between mineral and organic treatments for either time period.

Microbial relative abundance was similar across soils treated with organic and mineral fertilizers, with nearly 48% of the abundance consisting of Proteobacteria, followed by Actinobacteria (~18%), then Planctomycetes (9%) and all other remaining taxa (Figure 8).

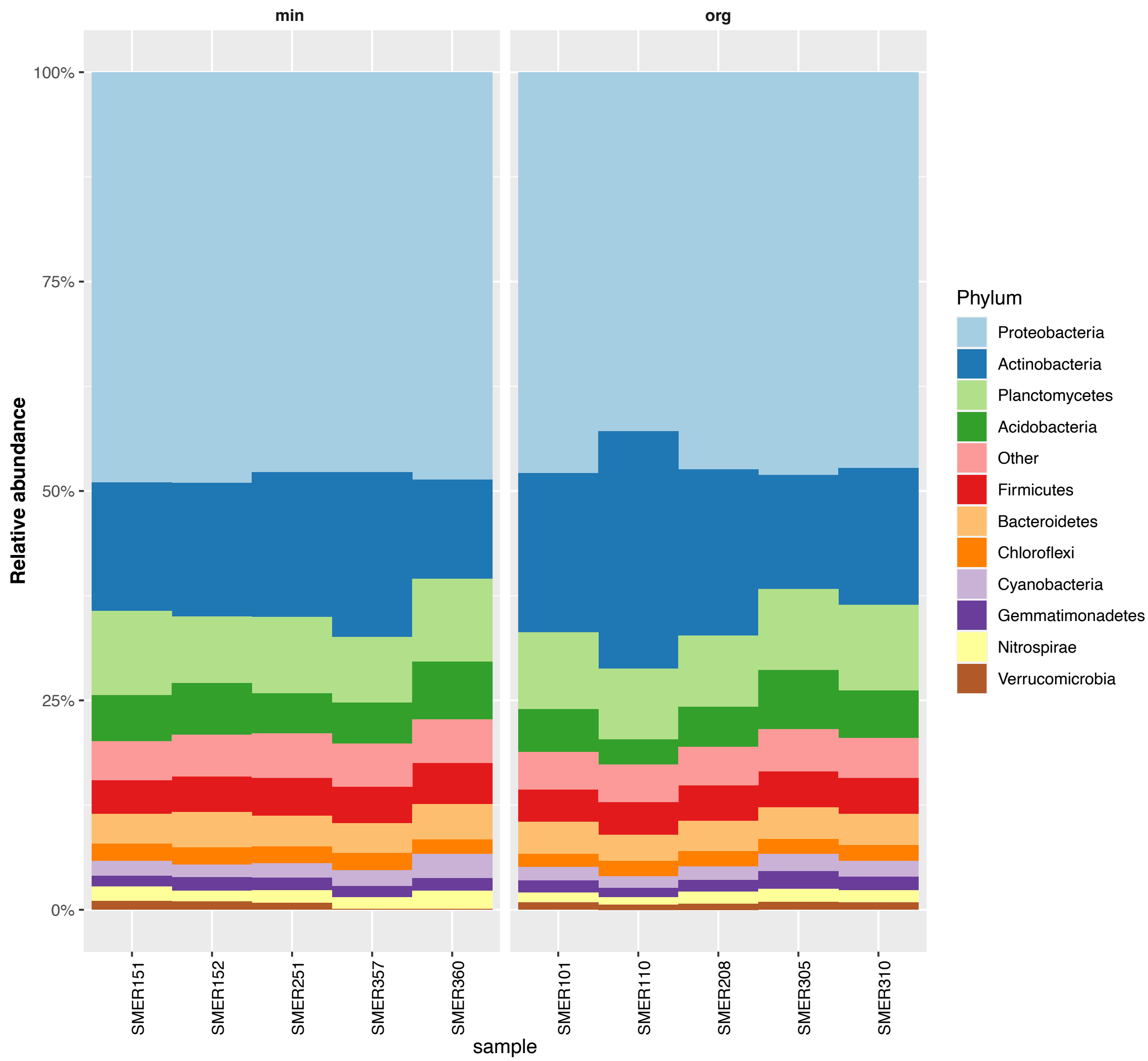


Figure 8. Relative abundance of soil microbes between the mineral and organic treatment, based on taxonomic classification of metagenomic data.

Microbial relative abundance in the native soil differed from grove soil with Actinobacteria comprising ~38%, followed by Proteobacteria (~37%), Acidobacteria (~4%), and all other remaining taxa (Figure 9).

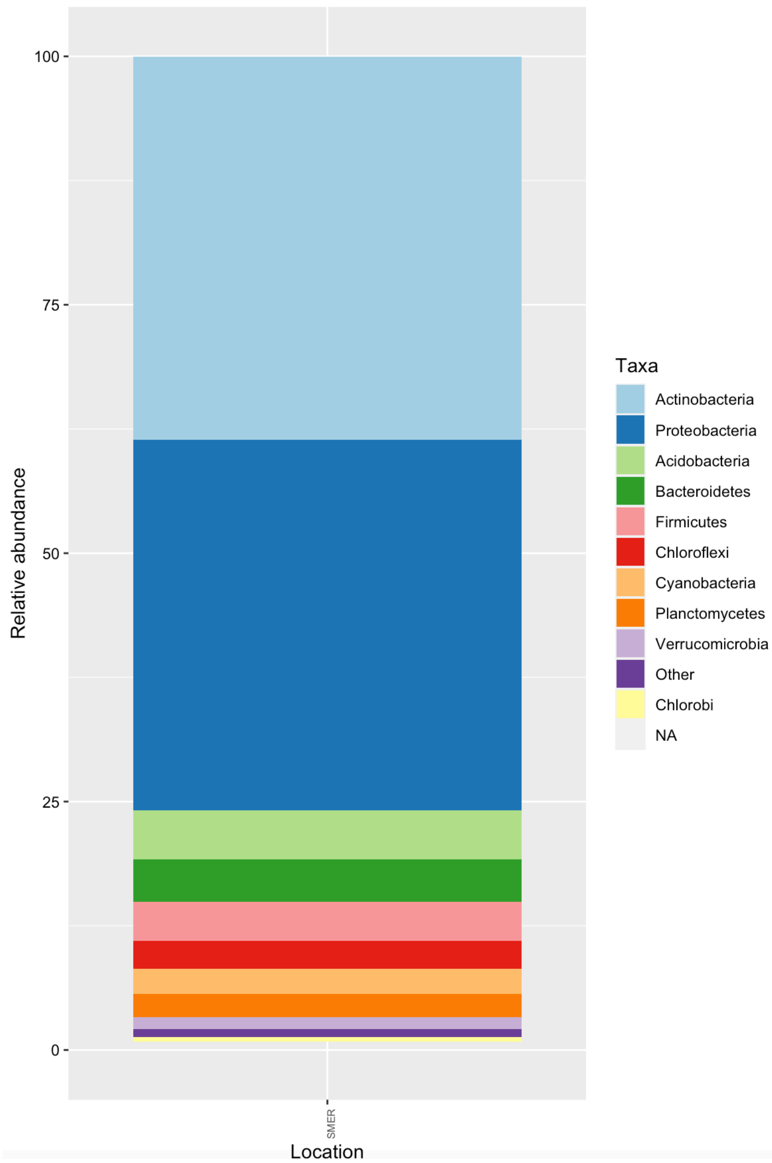


Figure 9. Relative abundance of soil microbes in native soils from SMER. Data obtained from Pérez Castro et al. (2019).

NMDS results indicate no difference in soil microbial composition when comparing soils treated with organic versus mineral fertilizer treatments (Figure 10).

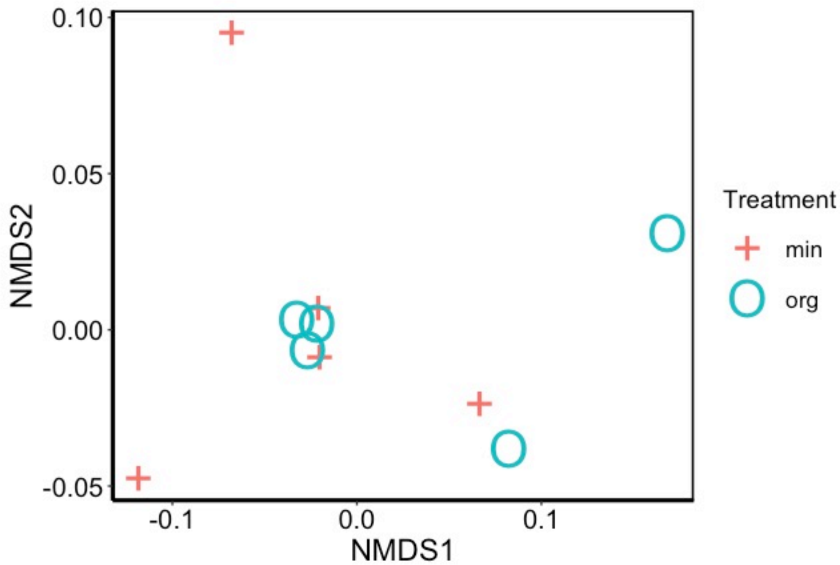


Figure 10. NMDS of soil microbes between the mineral and organic treatment. Random jiggle was applied to increase visibility of plot points.

The only nitrogen functions that were found to be different between soils treated with mineral versus organic fertilizers were ammonium oxidation and nitrate assimilatory reduction (Table 1).

Table 1. Average nitrogen functions between mineral and organic fertilizer treatments. Standard errors are shown in parentheses. Asterisks indicates a p-value of <0.05.

Nitrogen functions	Average mineral	Average organic
Ammonification	2.324 (0.325)	2.227 (0.204)
Ammonium oxidation*	**0.618 (0.074)**	**0.451 (0.048)**
Anaerobic ammonium oxidation	0.082 (0.021)	0.054 (0.011)
Denitrification	3.489 (0.209)	3.345 (0.170)
Nitrate assimilatory reduction*	**3.473 (0.162)**	**3.898 (0.063)**
Nitrate dissimilatory reduction	5.836 (0.333)	5.211 (0.293)
Nitrite assimilation	4.272 (0.131)	4.449 (0.144)
Nitrogen fixation	0.024 (0.006)	0.014 (0.003)
Urease	6.281 (0.214)	6.079 (0.259)

Discussion

Selladurai & Awachare (2020) determined that optimal N concentrations in leaves should be between 2 and 2.5 percent. The leaf N concentrations found in this study were within these optimal concentrations for both the mineral and organic treatments (Figure 2). The mineral fertilizer treatment indicates that the avocado trees have greater N uptake because leaves had higher percentages of N. However, since the organic fertilizer treatment also fell within the optimal range, it cannot be conclusively stated whether avocado trees benefit more from either fertilizer treatment regarding N uptake over a short period of time after fertilization.

Labeled glycine is retained in soil more than labeled inorganic N (Figure 3). This demonstrates that glycine would be available to plants for more extended periods of time (Aghaye Noroozlo et al., 2019). Future fertilizer applications for avocado groves should consider using more reduced forms of N (such as glycine) over traditional inorganic forms.

It appears that available N in the form of NH_4 is more easily retained in soil than in the form of NO_3, which leaches out of the soil in greater quantities (Figure 4), which aligns with previous findings (Mancino & Troll, 1990). This is true for both mineral and organic fertilizer treatments, indicating that neither one is superior for conserving N in the form of NO_3. There was no difference in net mineralization rates and net nitrification rates between mineral and organic fertilizer (Figures 5 & 6). The lack of difference in nitrification rates between these two types of fertilizer has been observed in a previous study (Zhang et al., 2012). However, emerging trends indicate that mineral fertilizer does increase both mineralization and net nitrification rates over the short-term, but over the long-term both fertilizer treatments have similar effects (Figure 5). Potential N leaching rates were not found to be different between mineral and organic fertilizer treatments (Figure 7), which is contrary to previous findings (Wang et al., 2015). This could be due to soil sampling shortly after initial fertilization, but after longer periods, this site could follow other findings and organic treatments could lead to a reduction in N losses.

This study did not find a difference in microbial communities between soils treated with mineral versus organic fertilizers (Figures 8 & 10). While this runs contrary to previous studies (Zhong et al., 2010; Li et al., 2015), it is possible that not enough time passed between fertilizer treatment initiation and soil sampling. However, the microbial communities between grove soils differed from the native soil with the grove soil showing an increase of Proteobacteria and Planctomycetes and a reduction of Actinobacteria. This indicates that the agriculture in the grove is shifting native microbial community populations, although the diverse microbial community in the grove soils suggests that tree crop agriculture does not adversely affect soil health. The decrease in relative abundance of Actinobacteria could suggest reduced environmental stress in the grove compared to native shrublands, as this phylum was associated with drought and climatic extremes (Pérez Castro et al., 2019). Findings related to ammonium oxidation and nitrate assimilatory reduction were found to be different between mineral and organic treatments (Table 1). The mineral treatment led to higher ammonium oxidation functions (Sun et al., 2022) and lower nitrate assimilatory reduction functions. Li et al. (2020) found that mineral fertilization led to increases in nitrate assimilatory reduction gene functions; however, Nardi et al. (2004) established that organic fertilizer treatments saw higher increases in nitrate reductase in comparison to mineral treatments.

There is little research examining whether mineral or organic fertilizers are better suited for avocado groves. Over a short period after fertilization treatments, it is hard to determine whether mineral or organic fertilizer treatments are superior in the growth of avocado crops. However, the fate of ^{15}N-labeled glycine compared to inorganic N forms over the course of this study indicates that mineral fertilizer was more rapidly lost from soil. Furthermore, the similarity of mineralization rates between mineral and organic fertilizer treatments after 5 months indicates that organic fertilizer may be a more efficient N application practice. It is possible that a combined approach of utilizing both organic and mineral fertilizers could be optimal for soil health, as the large organic matter inputs to the system from leaf litter could moderate the negative impacts of mineral fertilizer, such as soil degradation and reduced microbial diversity. Additionally, mineral fertilizer decreases microbial mining for nutrients from organic fertilizer, which sometimes leads to C loss via priming effects (Abdalla et al., 2022). This study sets a foundation for future research and serves as a baseline for short term fertilization results. Further research must be done on the SMER grove site to track long-term trends between mineral and organic fertilizer treatments. Avocados are a major California crop and recommendations for growers are vital given a growing need to adapt sustainable agricultural practices particularly with intensifying climate change effects.

Chapter 3: Long-term impacts of mineral versus organic fertilizers on trace metal storage in tomato and corn crops

Abstract

Soils are a primary sink and source of trace metals, especially in agricultural settings. Agricultural practices can lead to substantial heavy metal addition to soils through the use of fertilizers and pesticides. Whether soils serve as sinks or sources of trace metals depends on a wide range of factors that dictate their retention capacities for these metals. This study investigated the effect of long-term mineral versus compost fertilizer application on trace metal concentrations in soil through sequential extractions. DTPA extractions indicated a significant difference in bioavailability of Cu, Mn, and Zn between soils treated with compost versus mineral fertilizers. In soils treated with compost fertilizer, there was a slightly greater bioavailability of Cu, a substantially higher Zn bioavailability, and a lower Mn bioavailability than there was in soils treated with mineral fertilizers. There was no significant difference in the bioavailability of Fe between soils with different fertilizer treatments. BCR extractions indicated that there was no significant difference in concentrations of trace metals in the acid-extractable, reducible, and oxidizable soil components. There were significant differences in concentrations of Cr and Cu in the BCR residual components, as well as the total concentrations found through XRF. In all cases, concentrations were higher in the soils treated with compost fertilizer. Overall, both the DTPA and BCR extraction results suggest that organic fertilizer treatments increase bioavailability of Cu. Zn bioavailability was also found to increase with organic fertilizer per the DTPA extraction results and

the XRF data, and Cr bioavailability was found to increase with organic fertilizer per the BCR extraction results.

Introduction

Soils serve as a primary sink and source of trace metals, particularly in agricultural settings (Parelho et al., 2014). Agricultural practices can lead to heavy metal enrichment in soils because of liquid and solid manure, inorganic fertilizer, and pesticide applications (Parelho et al., 2014). Soil properties such as texture, organic matter content, ion-exchange capacity, oxide content, pH, specific surface area, and carbonate content dictate the retention capacity for trace metals (Parelho et al., 2014). Once the soil reaches this capacity it moves from serving as a sink to a source of trace metals and can lead to adverse human health effects while simultaneously disrupting ecosystem functioning (Parelho et al., 2014).

N, P, K, Ca, Mg, and S are considered to be vital macronutrients for plants; while Cu, Fe, Mn, and Zn are micronutrients essential for plant nutrition because they serve as co-factors for a variety of enzymes linked with the metabolism of diverse organic molecules (Dhaliwal et al., 2019). Micronutrients like Fe, Ni, Cu, and Zn are crucial for plants and soil microbes in trace amounts, but bioavailable versions of these nutrients are usually deficient in natural settings (Boiteau et al., 2018). Zn, in particular, is often deficient in soils across the United States (Zhang et al., 2016).

Typically, as soil pH rises, metal bioavailability decreases because of insoluble oxyhydroxide formations (Boiteau et al., 2018). Across the grasslands of the Midwestern United States, increased soil pH and calcium carbonate has been thought to cause iron deficiency chlorosis and diminished biomass yields in plants (Boiteau et al., 2018).

These grasslands are agriculturally important regions and serve as an important net sink of greenhouse gases which has advanced broad interest in comprehending how metabolic rates and ecosystem community dynamics are affected by nutrient and nutrient metal deficiencies (Boiteau et al., 2018).

Raised levels of trace metals in soil can negatively affect soil microbial communities (Khan et al., 2010), earthworms (Lourenço et al., 2011), nematodes (Santorufo et al., 2012), and plants (Nagajyoti et al., 2010). Trace metals in the soil can accumulate in less soluble forms, move to watersheds via leaching and erosion (Nziguheba & Smolders, 2008), or enter the food chain leading to toxicity in animals and humans (Parelho et al., 2014).

The addition of mineral fertilizers to agricultural soils can introduce trace metals which are of concern because of their possible environmental risk (Nziguheba & Smolders, 2008). Moreover, pure application of mineral fertilizers can lead to micronutrient deficiencies (Zhang et al., 2015). Mineral fertilizers contribute only macronutrients, forcing the plants to mine the soil for micronutrients which are then permanently removed from the system with the plants during harvest (Somani, 2016). The most common micronutrient deficiencies in crops are Zn deficiencies (Broadley et al., 2007). A lack of Zn can lead to several health complications both in humans (Hambidge & Krebs, 2007; Black et al., 2008) and plants (Broadley et al., 2007). Regular dietary intake of Zn is also necessary for livestock to meet all their physiological needs (Swain et al., 2016).

Application of various organic materials has been found to be an efficient approach of improving nutrient use efficiency and soil fertility (Dhaliwal et al., 2019).

Many cropping systems that have employed organic amendments have experienced high crop productivity because it affects physical and chemical soil properties which consequently influences crop micronutrient nutrition (Dhaliwal et al., 2019). Municipal solid waste compost has been found to enhance micronutrient complex formation, promote presence of useful soil organisms, decrease plant pathogens, improve soil porosity, and raise water holding capacity, soil buffering, and cation exchange capacity (Achiba et al., 2010). Organic fertilizers have also been used to make heavy metals unavailable to plants (Rai et al., 2004; Davis & Wilson, 2005; Gadepalle et al., 2007).

Regardless of these advantageous results, organic fertilizers can be a significant source of trace metal soil inputs in agriculture (Parelho et al., 2014). Organic fertilizers can also result in either the mobilization or immobilization of heavy metals for plant uptake (Davis & Wilson, 2005). Total concentrations of trace metals in soils with compost applications offer inadequate indications of their bioavailability, mobility, and reactivity capacity (Achiba et al., 2010).

Addition of organic fertilizers is linked to an increase in soil organic matter (SOM), which in turn affects the degree to which micronutrients are transferred to plants (Wajid et al., 2020). Previous studies have found that organic fertilizers can lead to an increased uptake of both micronutrients and trace metals in plants (Zhang et al., 2015; Wajid et al., 2020). In soil solution, a decrease in free cation concentrations could develop because of metals binding to organic matter; however, enhancement of metal phyto-availability at the root rhizosphere because of dissolution of these organo-metallic complexes can increase total dissolved ion concentration which is dependent upon mobility of metal-dissolved organic carbon (DOC) complexes and their dissociation kinetics (Dhaliwal et al., 2019). To a great degree, chelation of Zn and Fe with organic matter leads to enhancement of root accessible forms of these nutrients and also inhibits development of insoluble forms like soil carbonates and oxides (Dhaliwal et al., 2019).

The interactions between organic matter and metals are complex and can appear contradictory under different circumstances. Shuman (1975) found that the removal of SOM using sodium hypochlorite lowered Zn sorption capacity Zn binding energy. On the other hand, Trehan & Sekhon (1977) found that removal of SOM using traditional batch experiments increased Zn sorption on soil. Wajid et al. (2020) found that the application of manure increased the mobility of certain metals while decreasing the mobility of others. Some organic fertilizers, such as biochar, help reduce the mobility of heavy metals by altering soil redox potential (Gondek & Mierzwa-Hersztek, 2016). Barłóg et al. (2020) found that both mineral and organic fertilizers increased the concentrations of trace metals including both micronutrients (Zn, Cu, and Fe) and Cd, but that annual application of organic biogas-produced fertilizers did not lead to concentrations of toxic metals that were any higher than those generated by NPK fertilizers and other organic fertilizers, particularly cattle slurry fertilizers.

The purpose of this study was to investigate the effect of long-term mineral versus organic fertilizer application on trace metal concentrations in soil using sequential extractions to assess trace metal bioavailability. The hypotheses are 1) organic fertilizer additions will lead to a greater increase in bioavailability of trace metals to plants, and 2) organic fertilizer treatment will lead to more trace metal accumulation over mineral fertilizer treatment.

Methods

Field site

The field site is located at the Russell Ranch Sustainable Agricultural Research Facility, University of California, Davis (Davis, California, USA; 38°32′47″N 121°52′28″W) (Wang et al., under review). The region has a Mediterranean climate characterized by dry arid summers and wet winters (Wang et al., 2022). The soil is a Rincon silty clay loam (fine, smectitic, thermic Mollic Haploxeralfs, 20% sand, 49% silt, and 31% clay; 11 g C kg^{-1} C content; 1.30 g cm^{-3} bulk density) (Wang et al., 2022).

The cropping system considered in this experiment comprised a 2-yr rotation of processing tomato (*Lycopersicon esculentum* Mill.) and corn (*Zea mays* L.). Two fertility management systems were tested with equivalent N inputs, based on either: 1) mineral fertilizer (163 kg-N ha^{-1} $year^{-1}$, 50 kg-P ha^{-1} $year^{-1}$ and 50 kg-K ha^{-1} $year^{-1}$), or 2) poultry manure compost (4 Mg ha^{-1} applied yearly, including an incorporated winter cover crop for the first four years) (Wang et al., 2022).

Organic matter and pH

Organic matter content was evaluated through combustion (Salehi et al., 2011). The soil was tested using a soil water suspension ratio of 1:1.

Diethylenetriaminepentaacetic acid (DTPA) and Community Bureau of Reference (BCR) extractions

DTPA extractions were done according to Lindsay and Norvell (1978) and BCR extractions were done according to Sutherland and Tack (2002). The extractions were analyzed using PerkinElmer Inductively Coupled Plasma (ICP-OES). DTPA extractions were conducted to ascertain the availability of trace metals to plants, while BCR extractions were conducted to determine how trace metals were distributed across various chemical fractions. Both these methods were utilized because organic matter can either bind metals and make them unavailable or chelate them (Fan et al., 2016) which makes them easier to be accessed by plants (Zhang et al., 2016).

Soil elemental composition by X-ray fluorescence:

To expand the range of elements used in delineating patterns of soil fertility from Russell Ranch, Davis, soils were analyzed by energy-dispersive X-ray fluorescence (ED-XRF) using a XEPOS-HE spectrometer (Ametek Corporation, Berwyn, PA). Finely ball-milled samples (1.50-2.10g) were placed in a Teflon cup with a Prolene membrane suspended over the bottom of the cup. X-ray fluorescence spectra were acquired from four spots on each cup, count-rates averaged, and normalized using a built-in (“TQ-powders”) routine and normalizing to the Compton backscatter. The XRF detects elements with atomic numbers from Z=11 (Na) to Z=92 (U). QA/QC samples traceable to the National Bureau of Standards [NBS-1572; Gaithersburg MD] was measured on two occasions during XRF analyses, and values compared well to Certificate values, though Certificate values do not exist for all elements detectable by XRF.

Statistical analyses

Statistical analyses were conducted with R-Studio. ANOVAs were used to determine significance. Microsoft Excel was used to examine correlations.

Results

The DTPA extraction of trace metals indicated that there was a significant difference in concentrations of Cu, Mn, and Zn between soils treated with compost versus mineral fertilizers (Figure 1). In soils treated with compost fertilizer, there was a slightly greater concentration of Cu, a substantially higher Zn concentration, and a lower Mn concentration than there was in soils treated with mineral fertilizers. There was no significant difference in the quantities of Fe between soils with different fertilizer treatments. Of the trace metals identified through the DTPA extraction of soil treated with compost, Fe comprised approximately 45% of the extraction, followed by Mn at approximately 24%, then Zn at 19%, and finally Cu at 12%. Of those identified in the mineral soil, Fe comprised approximately 46%, Mn 33%, Cu 11%, and Zn was at about 10%.

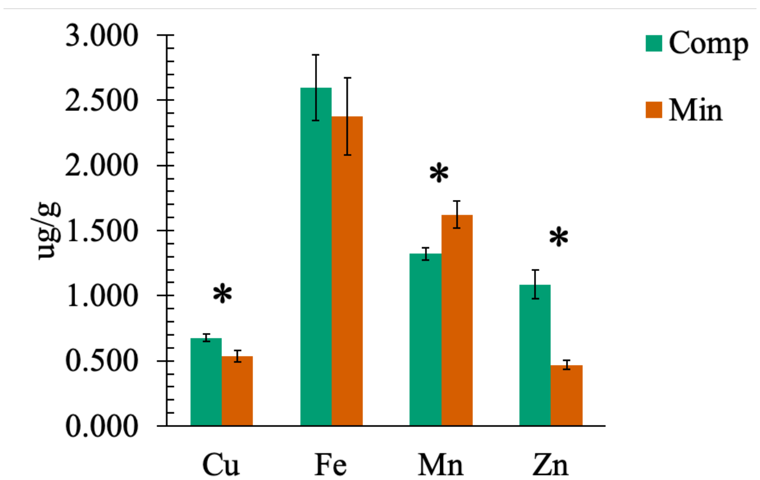


Figure 1. DTPA extraction of trace metals (Cu, Fe, Mn, and Zn) from Russell Ranch soil samples treated with compost and mineral fertilizers. Asterisks indicate statistical significance ($p < 0.05$).

The results of the BCR extraction indicated that there was no significant difference in concentrations of trace metals in the acid-extractable, reducible, and oxidizable components (Figure 2). There were significant differences in concentrations of Cr and Cu in the BCR residual components, as well as the total concentrations found through XRF. In all cases, concentrations were higher in the soils treated with compost fertilizer. Concentrations of Zn were only found to be significantly different between soil treatments through XRF, where it was also higher in soils treated with compost fertilizer.

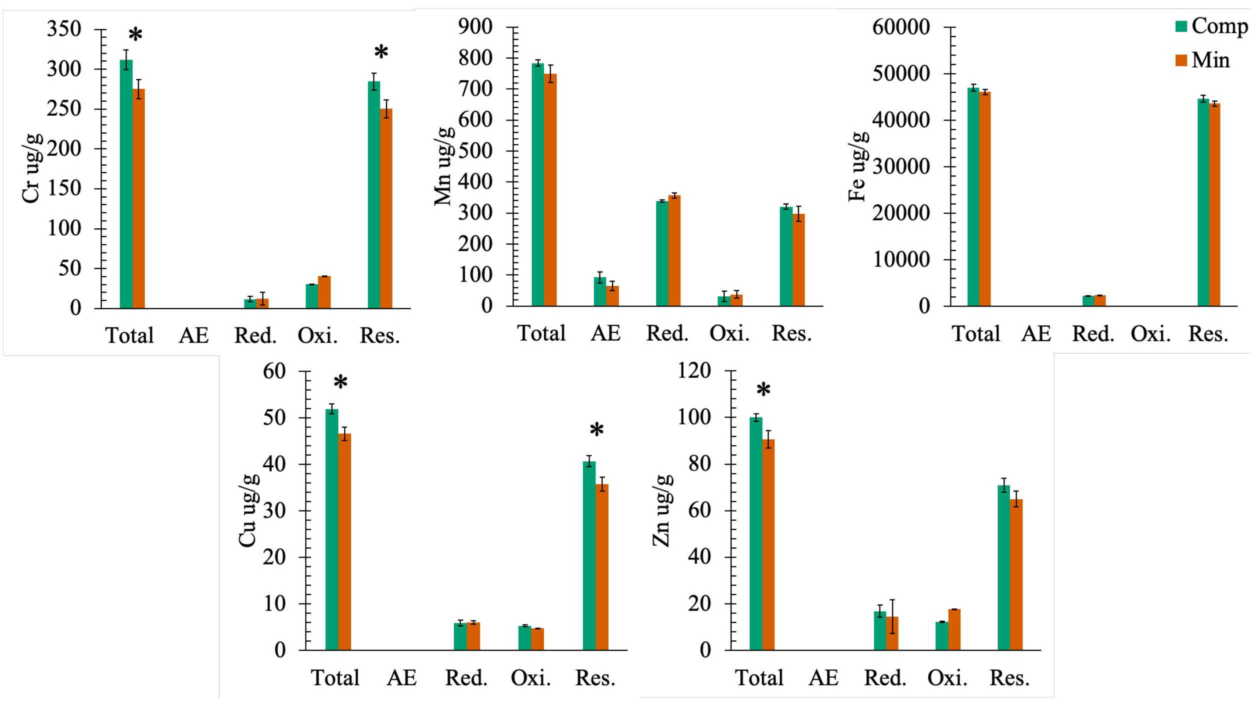


Figure 2. Comparison of BCR extraction-derived fraction concentrations (acid-extractable, reducible, oxidizable, and residual) of trace metals (Cr, Mn, Fe, Cu, and Zn) and XRF concentrations of the same trace metals. Asterisks indicate statistical significance ($p < 0.05$).

There was a positive correlation between the concentration of Zn found in the oxidizable fraction of the BCR extraction and the Zn concentration from the DTPA extraction, with a coefficient of determination of 0.7795 (Figure 3). This suggests that Zn was stored in the organic fraction of the soil. The was no correlation found between the amount of OM present in soil and soil pH (Figure 4).

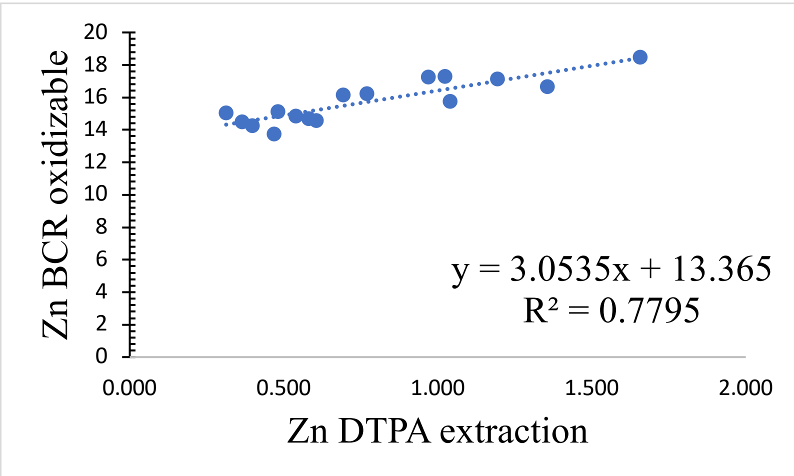


Figure 3. Correlation between DTPA extraction and BCR oxidizable fraction extraction of Zn (units in µg/g).

Discussion

The results of the DTPA extraction of trace metals supported the hypothesis that organic fertilizer additions will lead to a greater increase in bioavailability of trace metals to plants with regard to Cu and Zn; however, the results did not support the hypothesis with regard to Mn and Fe (Figure 1). Bioavailability of Cu and Zn was higher in soils treated with organic fertilizer. Conversely, greater concentrations of Mn were available to plants in soils treated with mineral fertilizers. The higher Mn concentrations found in soils treated with mineral fertilizer could be due to soil redox potential or soil pH (Appendix 1). There was no difference in bioavailability of Fe between fertilizer treatments. The results of Cu and Zn are corroborated by those from a previous study that found that trace metal concentrations were greater in maize grown from soils treated with organic fertilizer than they were in soils treated with mineral fertilizer (Wajid et al., 2020). The authors also found an increase in bioavailability of all other trace metals, but this is to be expected given that DTPA soil tests are only capable of detecting Zn, Fe, Mn, and Cu (Lindsay & Norvell, 1978; Dhaliwal et al., 2013). In order to detect Cr, other means of extraction were necessary. Although DTPA tests largely focus on inorganic compounds that are not part of the oxidizable pool, it should be noted that DTPA is more efficient than the acid-extractible component of the BCR extraction in pulling out available metals because it is not associated with iron oxides or organic matter.

BCR extraction results supported the hypothesis about greater trace metal accumulation in soils treated with organic fertilizer regarding Cr and Cu, but not Mn, Fe, or Zn, all three of which were found in similar concentrations between different fertilizer treatments. XRF data suggested similar results, with the addition of Zn, which was also found to be present in higher concentrations of soil treated with organic compost (Figure 2). Wajid et al. (2020) also found Cr and Cu concentrations to be higher in soils treated with organic fertilizers in comparison to those treated with mineral fertilizers, although they found all other trace metals to be higher as well.

Although there was a positive correlation between Zn concentrations in the DTPA and BCR extractions, the Zn concentrations were much higher in the BCR oxidizable fraction than those observed with the DTPA extraction (Figure 3). A possible explanation for this is that the majority of Zn is stored in the organic fraction of the soil. Another potential reason for the large difference in Zn concentrations found across these two extraction methods could be a difference in the quality of organic matter in different soil samples.

Overall, both the DTPA and BCR extraction results suggest that organic fertilizer treatments increase bioavailability of Cu. Zn bioavailability was also found to increase with organic fertilizer per the DTPA extraction results and the XRF data, and Cr bioavailability was found to increase with organic fertilizer per the BCR extraction results. Other studies have also found these micronutrients to have greater bioavailability in soils treated with organic fertilizer, although they have also detected increases in other micronutrients as well (Dhaliwal et al., 2013; Wajid et al., 2020). In contrast, one study found organic fertilizers reduced the bioavailability of some metals, including Cr (Alam et al., 2020).

Follow-up studies would benefit from qualitative analysis of the changes in organic matter between fertilizer treatments. Depending on the quality of the organic matter, Zn could be in a more loosely available complex form in certain samples than it is in others. It is possible that the availability of Fe for plant uptake is more dependent on soil redox potential. When the soil gets reduced, iron oxides could be liberated and thus, be more readily accessible to plants. Redox potential could also play a role in the availability of other metals in that as the soil is oxidized, organic matter occludes the metals, likely making them less available for plant uptake.

Chapter 3: Appendix

Table 1. Treatment plots with respective OM% and pH.

Plot	Treatment	OM%	pH
2019_12	Compost	3.78	7.53
2019_14	Compost	4.71	7.42
2019_2	Compost	4.68	7.46
2019_5	Compost	4.04	7.42
2020_12	Compost	3.58	7.66
2020_14	Compost	5.12	7.37
2020_2	Compost	3.85	7.34
2020_5	Compost	3.68	7.09
2019_13	Mineral	4.05	7.54
2019_3	Mineral	3.60	7.24
2019_7	Mineral	4.04	7.58
2019_9	Mineral	3.87	7.71
2020_13	Mineral	3.78	7.85
2020_3	Mineral	3.82	7.48
2020_7	Mineral	3.63	7.54
2020_9	Mineral	3.57	7.56

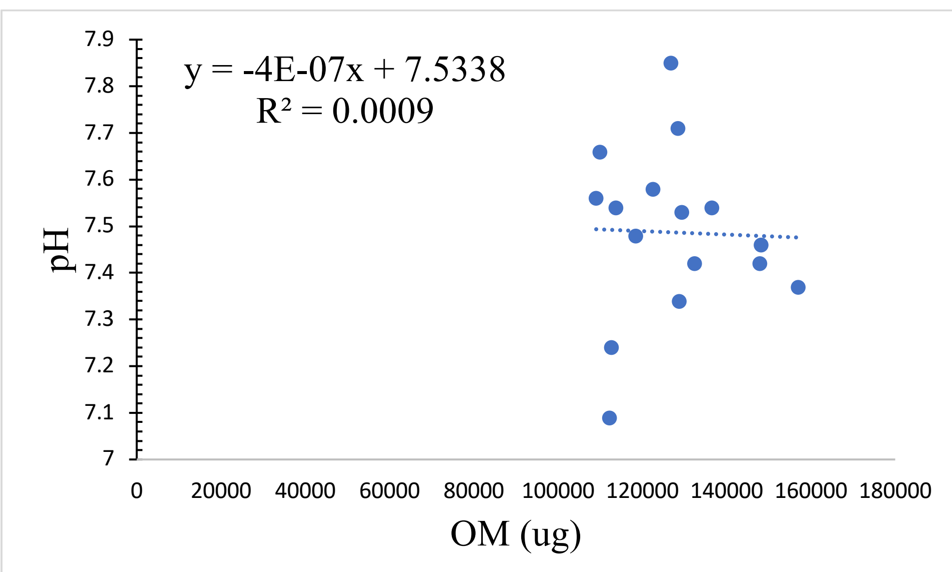


Figure 4. Correlation graph between OM (ug) and pH.

References

Abdalla, K., Sun, Y., Zarebanadkouki, M., Gaiser, T., Seidel, S., & Pausch, J. (2022). Long-term continuous farmyard manure application increases soil carbon when combined with mineral fertilizers due to lower priming effects. *Geoderma*, *428*, 116216.

Achiba, W. B., Lakhdar, A., Gabteni, N., Du Laing, G., Verloo, M., Boeckx, P., Van Cleemput, O., Jedidi, N., & Gallali, T. (2010). Accumulation and fractionation of trace metals in a Tunisian calcareous soil amended with farmyard manure and municipal solid waste compost. *Journal of Hazardous Materials, 176*: 99-108. doi: 10.1016/j.jhazmat.2009.11.004

Aghaye Noroozlo, Y., Souri, M. K., & Delshad, M. (2019). Effects of soil application of amino acids, ammonium, and nitrate on nutrient accumulation and growth characteristics of sweet basil. *Communications in Soil Science and Plant Analysis*, *50*(22), 2864–2872. https://doi.org/10.1080/00103624.2019.1689249

Aftab, T., & Hakeem, K. R. (Eds.). (2020). *Plant micronutrients: Deficiency and toxicity management*. Springer Nature.

Alam, M., Hussain, Z., Khan, A., Khan, M. A., Rab, A., Asif, M., Shah, M. A., & Muhammad, A. (2020). The effects of organic amendments on heavy metals bioavailability in mine impacted soil and associated human health risk. *Scientia Horticulturae*, *262*, 109067. https://doi.org/10.1016/j.scienta.2019.109067

Alkhaldi, A., Aldarir, A. N., Janat, M., Wahbi, A., & Arslan, A. (2012). Effect of regulated deficit irrigation and partial root-zone drying on some quantitative indicators and the efficiency of adding nitrogen fertilizer to (Zea mays L.) by using n15 Isotope. *Am-Eu J Agric Env Sci*, *12*, 1223-1235.

Arkin, A. P., Cottingham, R. W., Henry, C. S., Harris, N. L., Stevens, R. L., Maslov, S., Dehal, P., Ware, D., Perez, F., Canon, S., Sneddon, M. W., Henderson, M. L., Riehl, W. J., Murphy-Olson, D., Chan, S. Y., Kamimura, R. T., Kumari, S., Drake, M. M., Brettin, T. S., … Yu, D. (2018). KBase: The United States Department of Energy Systems Biology Knowledgebase. *Nature Biotechnology*, *36*(7), 566–569. https://doi.org/10.1038/nbt.4163

Babbar, L. I., & Zak, D. R. (1994). Nitrogen cycling in coffee agroecosystems: net N mineralization and nitrification in the presence and absence of shade trees. *Agriculture, ecosystems & environment*, *48*(2), 107-113.

Bafaro, E., Liu, Y., Xu, Y., & Dempski, R. E. (2017). The emerging role of zinc transporters in cellular homeostasis and cancer. Signal transduction and targeted therapy, 2(1), 1-12.

Bai, X., Huang, Y., Ren, W., Coyne, M., Jacinthe, P., Tao, B., Hui, D., Yang, J., & Matocha, C. (2019). Responses of soil carbon sequestration to climate-smart agriculture practices: A meta-analysis. *Global Change Biology*, *25*(8), 2591–2606. https://doi.org/10.1111/gcb.14658

Barłóg, P., Hlisnikovský, L., & Kunzová, E. (2020). Concentration of trace metals in winter wheat and spring barley as a result of digestate, cattle slurry, and mineral fertilizer application. *Environmental Science and Pollution Research*, *27*(5), 4769–4785. https://doi.org/10.1007/s11356-019-07304-2

Bhattacharya, R., V. Prakash, S. Kundu, A. K. Srivasta, H. S. Gupta, and S. Mitra. 2010. Long term effects of fertilization on carbon and nitrogen sequestration and aggregate associated carbon and nitrogen in the Indian sub-Himalayas. *Nutrient Cycling in Agroecosystems*, 86: 1-16.

Behera, S. K., Shukla, A. K., & Patra, A. K. (2019). Best micronutrient management practices. Indian Farming, 69(04), 11-16.

Black, R. E., Allen, L. H., Bhutta, Z. A., Caulfield, L. E., de Onis, M., Ezzati, M., Mathers, C., & Rivera, J. (2008). Maternal and child undernutrition: Global and regional exposures and health consequences. *The Lancet*, *371*(9608), 243–260. https://doi.org/10.1016/S0140-6736(07)61690-0

Boiteau, R. M., Shaw, J. B., Pasa-Tolic, L., Koppenaal, D. W., & Jansson, J. K. (2018). *Soil Biology and Biochemistry, 120*: 283-291. https://doi.org/10.1016/j.soilbio.2018.02.018

Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I., & Lux, A. (2007). Zinc in plants. *New Phytologist*, *173*(4), 677–702. https://doi.org/10.1111/j.1469-8137.2007.01996.x

Buckeridge, K. M., Creamer, C., & Whitaker, J. (2022). Deconstructing the microbial necromass continuum to inform soil carbon sequestration. *Functional Ecology*, *36*(6), 1396-1410.

Bucka, F. B., Kölbl, A., Uteau, D., Peth, S., & Kögel-Knabner, I. (2019). Organic matter input determines structure development and aggregate formation in artificial soils. *Geoderma*, *354*, 113881.

Canfield, D. E., Glazer, A. N., & Falkowski, P. G. (2010). The evolution and future of Earth’s nitrogen cycle. *science*, *330*(6001), 192-196.

California Department of Food and Agriculture. (2016). Citrus production in California [PDF document]. Retrieved from https://apps1.cdfa.ca.gov/FertilizerResearch/docs/Citrus_Production_CA.pdf

California Department of Food and Agriculture. (2013). California agricultural statistics review 2012-2013. Retrieved from https://www.cdfa.ca.gov/statistics/pdfs/2013/fruitandnut.pdf

California Department of Food and Agriculture. (n.d.). Avocado production in California [PDF document]. Retrieved from https://apps1.cdfa.ca.gov/FertilizerResearch/docs/Avocado_Production_CA.pdf

Canfield, D. E., Glazer, A. N., & Falkowski, P. G. (2010). The evolution and future of Earth’s nitrogen cycle. *Science*, *330*(6001), 192-196.

Chapin, F. S., Matson, P. A., & Mooney, H. A. (2002). Carbon input to terrestrial ecosystems. *Principles of terrestrial ecosystem ecology*, 97-122.

Chavarria, D. N., Pérez-Brandan, C., Serri, D. L., Meriles, J. M., Restovich, S. B., Andriulo, A. E., ... & Vargas-Gil, S. (2018). Response of soil microbial communities to agroecological versus conventional systems of extensive agriculture. Agriculture, Ecosystems & Environment, 264, 1-8.

Chen, H., D. Li, G. A. Gurmesa, L. Li, W. Zhang, H. Fang, and J. Mo. 2015. Effects of nitrogen deposition on carbon cycle in terrestrial ecosystems of China: A meta-analysis. *Environmental Pollution*, 206: 352-360.

Compton, J. E. and R. D. Boone. 2000. Long-term impacts of agriculture on soil carbon and nitrogen in New England forests. *Ecology*, 81: 2314-2330.

Crowley, D. E., Smith, W., Faber, B., & Manthey, J. A. (1996). Zinc fertilization of avocado trees. HortScience, 31(2), 224-229.

Davidson, E. A., de Carvalho, C. J. R., Figueira, A. M., Ishida, F. Y., Ometto, J. P. H., Nardoto, G. B., ... & Martinelli, L. A. (2007). Recuperation of nitrogen cycling in Amazonian forests following agricultural abandonment. *Nature*, *447*(7147), 995-998.

Davis, J. G., & Wilson, C. R. (2005). Colorado State University cooperative extension-horticulture. Bulletin 7, 235.

Das, S., & Green, A. (2016). Zinc in crops and human health. Biofortification of food crops, 31-40.

Dhaliwal, S. S., Manchanda, J. S., Walia, S. S., & Dhaliwal, M. K. (2013). Differential response of manures in transformation of DTPA and total zinc and iron in rice transplanted on light textured soils of Punjab. *International Journal of Science, Environment and Technology*, *2*(3), 300–312.

Dhaliwal, S. S., Naresh, R. K., Mandal, A., Singh, R., & Dhaliwal, M. K. (2019). Dynamics and transformations of micronutrients in agricultural soils as influenced by organic matter build-up: A review. *Environmental and Sustainability Indicators*. https://doi.org/10.1016/j.indic.2019.100007

EPA. (2015). Avocado production in California. Retrieved from https://www.epa.gov/sites/default/files/2015-09/ca_avocado.doc

Fan, T.-T., Wang, Y.-J., Li, C.-B., He, J.-Z., Gao, J., Zhou, D.-M., Friedman, S. P., & Sparks, D. L. (2016). Effect of Organic Matter on Sorption of Zn on Soil: Elucidation by Wien Effect Measurements and EXAFS Spectroscopy. *Environmental Science & Technology*, *50*(6), 2931–2937. https://doi.org/10.1021/acs.est.5b05281

Fierer, N. 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews – Microbiology*, 15: 579-590.

Fikry, A. M., Abou Sayed-Ahmed, T. A. M., Mohsen, F. S., & Ibrahim, M. M. (2020). Effect of Nitrogen fertilization through inorganic, organic and biofertilizers sources on vegetative growth, yield and nutritional status in Murcott Tangerine trees. *Plant Archives*, *20*(1), 1859-1868.

Gadepalle, V. P., Ouki, S. K., Herwijnen, R. V., & Hutchings, T. (2007). Immobilization of heavy metals in soil using natural and waste materials for vegetation establishment on contaminated sites. *Soil & Sediment Contamination 16*(2), 233–251.

Galloway, J. N., F. J. Dentener, D. G. Capone, E.W. Boyer, R. W. Howarth, S. P Seitzinger, G. P. Asner, C. C. Cleveland, P. A. Green, E. A. Holland, D. M. Karl,

A. F. Michaels, J. H. Poter, A. R. Townsend, & C. J. Vorosmarty. (2004). Nitrogen cycles: past, present, and future. *Biogeochemistry*, *70*, 153-226.

Gama-Rodrigues, A. C. (2011). Soil organic matter, nutrient cycling and biological dinitrogen-fixation in agroforestry systems. *Agroforestry Systems*, *81*(3), 191–193. https://doi.org/10.1007/s10457-011-9372-9

Gondek, K., & Mierzwa-Hersztek, M. (2016). Effect of low-temperature biochar derived from pig manure and poultry litter on mobile and organic matter-bound forms of Cu, Cd, Pb and Zn in sandy soil. *Soil Use and Management*, *32*(3), 357–367. https://doi.org/10.1111/sum.12285

Gougoulias, C., Clark, J. M., & Shaw, L. J. (2014). The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems. *Journal of the Science of Food and Agriculture*, *94*(12), 2362-2371.

Groenenberg, J. E., Römkens, P. F., Zomeren, A. V., Rodrigues, S. M., & Comans, R. N. (2017). Evaluation of the single dilute (0.43 M) nitric acid extraction to determine geochemically reactive elements in soil. *Environmental science & technology*, *51*(4), 2246-2253.

Grunwald, D., M. Kaiser, and B. Ludwig. 2016. Effect of biochar and organic fertilizers on C mineralization and macro-aggregate dynamics under different incubation temperatures. *Soil & Tillage Research*, 164: 11-17.

Hambidge, K. M., & Krebs, N. F. (2007). Zinc deficiency: A special challenge. *The Journal of Nutrition, 137*(4), 1101–1105. https://doi.org/10.1093/jn/137.4.1101

Hänsch, R., & Mendel, R. R. (2009). Physiological functions of mineral micronutrients (cu, Zn, Mn, Fe, Ni, Mo, B, cl). *Current opinion in plant biology*, *12*(3), 259-266.

Hartmann, M., Frey, B., Mayer, J., Mäder, P., & Widmer, F. (2015). Distinct soil microbial diversity under long-term organic and conventional farming. The ISME journal, 9(5), 1177-1194.

Hietz, P., Dünisch, O., & Wanek, W. (2010). Long-term trends in nitrogen isotope composition and nitrogen concentration in Brazilian rainforest trees suggest changes in nitrogen cycle. *Environmental science & technology*, *44*(4), 1191-1196.

Hosomi, M., & Sudo, R., (1986). Simultaneous determination of total nitrogen and total phosphorus in freshwater samples using persulfate digestion. *International Journal of Environmental Studies*, *27*(3-4), 267-275.

Howe, J. A., & Smith, A. P. (2021). The soil habitat. In *Principles and Applications of Soil Microbiology* (3rd ed., pp. 23–55). Elsevier.

Huang, S., X. Peng, Q. Huang, and W. Zhang. 2010. Soil aggregation and organic carbon fractions affected by long-term fertilization in a red soil of subtropical China. *Geoderma*, 154: 364-369.

Imtiaz, M., Rashid, A., Khan, P., Memon, M. Y., & Aslam, M. (2010). The role of micronutrients in crop production and human health. Pakistan *Journal of Botany*, *42*(4), 2565-2578.

Jia, G., E. Shevliakova, P. Artaxo, N. De Noblet-Ducoudré, R. Houghton, J. House, K. Kitajima, C. Lennard, A. Popp, A. Sirin, R. Sukumar, L. Verchot, 2019: Land–climate interactions*. In: Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems* [P.R. Shukla, J. Skea, E. Calvo Buendia, V. Masson-Delmotte, H.-O. Pörtner, D.C. Roberts, P. Zhai, R. Slade, S. Connors, R. van Diemen, M. Ferrat, E. Haughey, S. Luz, S. Neogi, M. Pathak, J. Petzold, J. Portugal Pereira, P. Vyas, E. Huntley, K. Kissick, M, Belkacemi, J. Malley, (eds.)]. In press.

Jones, D. L., Cross, P., Withers, P. J. A., DeLuca, T. H., Robinson, D. A., Quilliam, R. S., Harris, I. M., Chadwick, D. R., & Edwards-Jones, G. (2013). Nutrient stripping: The global disparity between food security and soil nutrient stocks. *Journal of Applied Ecology*, *50*(4), 851–862. https://doi.org/10.1111/1365-2664.12089

Kantola, I. B., Masters, M. D., Beerling, D. J., Long, S. P., & DeLucia, E. H. (2017). Potential of global croplands and bioenergy crops for climate change mitigation through deployment for enhanced weathering. Biology letters, 13(4), 20160714.

Khalsa, S. D. S., HARTZ, T. K., & Brown, P. H. (2018). Principles of Nitrogen Cycling and Management.

Khan, S., Hesham, A.E-L., Qiao, M., Rehman, S., & He, J. Z. (2010). Effects of Cd and Pb on soil microbial community structure and activities. *Environmental Science and Pollution Research, 17*: 288–296. http://dx.doi.org/10.1007/s11356-009-0134-4

Kibblewhite, M. G., Ritz, K., & Swift, M. J. (2008). Soil health in agricultural systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1492), 685–701. https://doi.org/10.1098/rstb.2007.2178

Lal, R. (2004). Soil carbon sequestration to mitigate climate change. *Geoderma*, *123*(1-2), 1-22.

Li, J., Cooper, J. M., Lin, Z., Li, Y., Yang, X., & Zhao, B. (2015). Soil microbial community structure and function are significantly affected by long-term organic and mineral fertilization regimes in the North China Plain. *Applied Soil Ecology*, *96*, 75–87. https://doi.org/10.1016/j.apsoil.2015.07.001

Li, Y., Tremblay, J., Bainard, L. D., Cade-Menun, B., & Hamel, C. (2020). Long-term effects of nitrogen and phosphorus fertilization on soil microbial community structure and function under continuous wheat production. *Environmental Microbiology*, *22*(3), 1066-1088.

Liao, J. D., T. W. Boutton, and J. D. Jastrow. 2006. Storage and dynamics of carbon and nitrogen in soil physical fractions following woody plant invasion of grassland. *Soil Biology and Biochemistry*, 38: 3184-3196.

Lindsay, W.L., & Norvell, W.A. (1978). Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper. *Soil Science Society of America Journal, 42*: 421-428. https://doi.org/10.2136/sssaj1978.03615995004200030009x

Lipson, D. A., Jha, M., Raab, T. K., & Oechel, W. C. (2010). Reduction of iron (III) and humic substances plays a major role in anaerobic respiration in an Arctic peat soil. *Journal of Geophysical Research: Biogeosciences*, *115*(G4).

Lobell, D. B., Field, C. B., Cahill, K. N., & Bonfils, C. (2006). Impacts of future climate change on California perennial crop yields: Model projections with climate and crop uncertainties. *Agricultural and Forest Meteorology*, *141*(2-4), 208-218.

Lourenço, J. I., Pereira, R. O., Silva, A. C., Morgado, J. M., Carvalho, F. P., Oliveira, J. M., Malta, M. P., Paiva, A. A., Mendo, S. A., & Gonçalves, F. J. (2011). Genotoxic endpoints in the earthworms sub-lethal assay to evaluate natural soils contaminated by metals and radionuclides. *Journal of Hazardous Materials, 186*: 788–795. http://dx.doi.org/10.1016/j. jhazmat.2010.11.073

Lu, M., X. Zhou, Y. Luo, Y. Yang, C. Fang, J. Chen, and B. Li. 2011. Minor stimulation of soil carbon storage by nitrogen addition: A meta-analysis. *Agriculture, Ecosystems and Environment*, 140: 234–244.

Mancino, C. F., & Troll, J. (1990). Nitrate and ammonium leaching losses from n fertilizers applied to `Penncross' creeping bentgrass. *HortScience*, *25*(2), 194–196. https://doi.org/10.21273/HORTSCI.25.2.194

Mattos-Jr, D., Huber, L. N., Petená, G., Bortoloti, G. A., Hippler, F. W. R., & Boaretto, R. M. (2023). Biochemical and anatomical aspects of copper deficiency induced by high nitrogen supply in citrus. *Plant and Soil*. https://doi.org/10.1007/s11104-023-05899-7

Mellado-Vasquez, P. G., M. Lange, and G. Gleixner. 2019. Soil microbial communities and their carbon assimilation are affected by soil properties and season but not by plants differing in their photosynthetic pathways (C3 vs. C4). *Biogeochemistry*, 142: 175-187.

Mendes, L. W., M. J. L. Brossi, E. E. Kuramae, and S. M. Tsai. 2015. Land-use system shapes soil bacterial communities in Southeastern Amazon region. *Applied Soil Ecology*, 95: 151-160.

Montanaro, G., Xiloyannis, C., Nuzzo, V., & Dichio, B. (2017). Orchard management, soil organic carbon and ecosystem services in Mediterranean fruit tree crops. Scientia Horticulturae, 217, 92-101.

Nagajyoti, P. C., Lee, K. D., & Sreekanth, T. V. M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental Chemistry Letters, 8*: 199–216. http://dx.doi.org/10.1007/s10311-010- 0297-8

Nardi, S., Morari, F., Berti, A., Tosoni, M., & Giardini, L. (2004). Soil organic matter properties after 40 years of different use of organic and mineral fertilisers. *European Journal of Agronomy*, *21*(3), 357-367.

Nömmik, H. (1966). The uptake and translocation of fertilizer N15 in young trees of Scots pine and Norway spruce.

Nziguheba, G., & Smolders, E. Inputs of trace elements in agricultural soils via phosphate fertilizers in European countries. (2008). *Science of the Total Environment, 390*: 53-57. doi: 10.1016/j.scitotenv.2007.09.031

Oertel, C., Matschullat, J., Zurba, K., Zimmermann, F., & Erasmi, S. (2016). Greenhouse gas emissions from soils—A review. *Geochemistry*, *76*(3), 327-352.

Parelho, C., Rodrigues, A. S., Cruz, J. V., & Garcia, P. (2014). Linking trace metals and agricultural land use in volcanic soils--a multivariate approach. *Science of the Total Environment, 496*: 241-247. doi: 10.1016/j.scitotenv.2014.07.053

Patnaik, S. (1965, January). N15 tracer studies on the utilization of fertilizer nitrogen by rice in relation to time of application. In *Proceedings/Indian Academy of Sciences* (Vol. 61, No. 1, pp. 31-38). New Delhi: Springer India.

Pérez Castro, S., Cleland, E. E., Wagner, R., Sawad, R. A., & Lipson, D. A. (2019). Soil microbial responses to drought and exotic plants shift carbon metabolism. *The ISME Journal*, *13*(7), 1776-1787.

Pestana, M., Correia, P. J., David, M., Abadía, A., Abadía, J., & de Varennes, A. (2011). Response of five citrus rootstocks to iron deficiency. *Journal of Plant Nutrition and Soil Science*, *174*(5), 837–846. https://doi.org/10.1002/jpln.201000341

Rai, U. N., Pandey, K., Sinha, S., Singh, A., Saxena, R., & Gupta, D. K. (2004). Revegetating fly ash landfills with *Prosopis juliflora* L.: Impact of different amendments and Rhizobium inoculation. *Environmental International, 30*(3), 293–300.

Ramírez-Gil, J. G., Castañeda-Sánchez, D., & Morales-Osorio, J. G. (2021). Edaphic factors associated with the development of avocado wilt complex and implementation of a GIS tool for risk visualization. *Scientia Horticulturae*, *288*, 110316. https://doi.org/10.1016/j.scienta.2021.110316

Reddy, M., Moodley, R., & Jonnalagadda, S. B. (2014). Elemental uptake and distribution of nutrients in avocado mesocarp and the impact of soil quality.

Environmental Monitoring and Assessment, *186*(7), 4519–4529. https://doi.org/10.1007/s10661-014-3716-7

Salazar-García, S. (1999). Iron nutrition and deficiency: A review with emphasis. *Revista Chapingo Serie Horticultura*, *5*(2), 67–76.

Salehi, M.H., Hashemi Beni, O., Beigi Harchegani, H., Esfandiarpour Borujeni, I., & Motaghian, H.R. (2011). Refining Soil Organic Matter Determination by Loss-on-Ignition. *Pedosphere, 21*: 473–482.

Santorufo, L., Van Gestel, C., Rocco, A., & Maisto, G. (2012). Soil invertebrates as bioindicators of urban soil quality. *Environmental Pollution, 161*: 57–63. http://dx.doi.org/10.1016/j.envpol.2011. 09.042

Schlesinger, W. H. and J. A. Andrews. 2000. Soil respiration and the global carbon cycle. *Biogeochemistry*, 48: 7-20.

Scow, K. M. (1997). Soil microbial communities and carbon flow in agroecosystems. In *Ecology in Agriculture* (pp. 367–413). Elsevier. https://doi.org/10.1016/B978-012378260-1/50012-9

Selladurai, R., & Awachare, C. M. (2020). Nutrient management for avocado (*Persea americana* miller). *Journal of Plant Nutrition*, *43*(1), 138–147. https://doi.org/10.1080/01904167.2019.1659322

Shukla, A. K., Behera, S. K., Prakash, C., Patra, A. K., Rao, C. S., Chaudhari, S. K., ... & Green, A. (2021). Assessing multi-micronutrients deficiency in agricultural soils of India. *Sustainability*, *13*(16), 9136.

Shuman, L. (1975). The effect of soil properties on zinc adsorption by soils. *Soil Science Society of America Journal, 39*, 454–458.

Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil Tillage and Research*, 79: 7-31.

Smith, D. R., Livingston, S. J., Zuercher, B. W., Larose, M., Heathman, G. C., & Huang, C. (2008). Nutrient losses from row crop agriculture in Indiana. *Journal of Soil and Water Conservation*, *63*(6), 396–409. https://doi.org/10.2489/jswc.63.6.396

Smith, K. A., and C. E. Mullins. 1991. Soil Analysis. Physical Methods. Marcel Dekker, New York, p. 620.

Somani, L. L. (2016). *Micronutrients for soil and plant health*. Agrotech Publishing Academy.

Stark, J. M., & Hart, S. C. (1996). Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. *Soil Science Society of America Journal*, *60*(6), 1846-1855.

Strickland, T. C., & Sollins, P. (1987). Improved method for separating light-and heavy-fraction organic material from soil. *Soil Science Society of America Journal*, *51*(5), 1390-1393.

Sun, G., Sun, M., Luo, Z., Li, C., Xiao, X., Li, X., Zhong, J., Wang, H., & Nie, S. (2022). Effects of different fertilization practices on anammox activity, abundance, and community compositions in a paddy soil. *Soil Ecology Letters*, *4*(3), 254–263. https://doi.org/10.1007/s42832-021-0103-5

Sun, W., Canadell, J. G., Yu, L., Yu, L., Zhang, W., Smith, P., Fischer, T., & Huang, Y. (2020). Climate drives global soil carbon sequestration and crop yield changes under conservation agriculture. *Global Change Biology*, *26*(6), 3325–3335. https://doi.org/10.1111/gcb.15001

Sutherland, R., & Tack, F. (2002). Determination of Al, Cu, Fe, Mn, Pb and Zn in certified reference materials using the optimized BCR sequential extraction procedure. *Analytica Chimica Acta, 454*: 249–257.

Swain, P. S., Rao, S. B. N., Rajendran, D., Dominic, G., & Selvaraju, S. (2016). Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. *Animal Nutrition*, *2*(3), 134–141. https://doi.org/10.1016/j.aninu.2016.06.003

Tang, Z., X. Sun, Z. Luo, N. He, and O. J. Sun. 2018. Effects of temperature, soil substrate, and microbial community on carbon mineralization across three climatically contrasting forest sites. *Ecology and Evolution*, 8: 879-891.

Tebrügge, F. (2003). No-tillage visions-protection of soil, water and climate and influence on management and farm income. Conservation agriculture: environment, farmers experiences, innovations, socio-economy, policy, 327-340.

Toselli, M., Baldi, E., Cavani, L., Mazzon, M., Quartieri, M., Sorrenti, G., & Marzadori, C. (2019). Soil-plant nitrogen pools in nectarine orchard in response to long-term compost application. Science of the Total Environment, 671, 10-18.

Trehan, S. P., & Sekhon, G. S. (1977). Effect of clay, organic matter and $CaCO_3$ content on zinc adsorption by soils. *Plant and Soil, 46*, 329−336.

Tripathi, D. K., Singh, S., Singh, S., Mishra, S., Chauhan, D. K., & Dubey, N. K. (2015). Micronutrients and their diverse role in agricultural crops: advances and future prospective. *Acta Physiologiae Plantarum*, *37*, 1-14.

Tripathi, R., A. K. Nayak, P. Bhattacharya, A. K. Shukla, M. Shahid, R. Raja, B. B. Panda, S. Mohanty, A. Kumar, and V. K. Thilagam. (2014). Soil aggregation and distribution of carbon and nitrogen in different fractions after 41 years long-term fertilizer experiment in tropical rice–rice system. *Geoderma*, 213: 280-286.

Trumbore, S. (2006). Carbon respired by terrestrial ecosystems–recent progress and challenges. *Global Change Biology*, *12*(2), 141-153.

Vitousek, P. M., S. Hattenschwiler, L. Olander, and S. Allison. 2002. Nitrogen and nature. *Ambio*, 31: 97-101.

USDA. 2022. https://www.ers.usda.gov/topics/natural-resources-environment/climate-change/

Vitousek, P. M., Hättenschwiler, S., Olander, L., & Allison, S. (2002). Nitrogen and nature. *AMBIO: A Journal of the Human Environment*, *31*(2), 97-101.

Wajid, K., Ahmad, K., Khan, Z. I., Nadeem, M., Bashir, H., Chen, F., & Ugulu, I. (2020). Effect of organic manure and mineral fertilizers on bioaccumulation and translocation of trace metals in maize. *Bulletin of Environmental Contamination and Toxicology, 104*(5): 649–657. https://doi.org/10.1007/s00128-020-02841-w

Wang, D., Lin, J. Y., Sayre, J. M., Schmidt, R., Fonte, S. J., Rodrigues, J. L., & Scow, K. M. (2022). Compost amendment maintains soil structure and carbon storage by increasing available carbon and microbial biomass in agricultural soil–A six-year field study. *Geoderma*, *427*, 116117.

Wang, J., Zhu, B., Zhang, J., Müller, C., & Cai, Z. (2015). Mechanisms of soil N dynamics following long-term application of organic fertilizers to subtropical rain-fed purple soil in China. *Soil Biology and Biochemistry*, *91*, 222-231.

Wei, Z., Ying, H., Guo, X., Zhuang, M., Cui, Z., & Zhang, F. (2020). Substitution of mineral fertilizer with organic fertilizer in maize systems: A meta-analysis of reduced nitrogen and carbon emissions. *Agronomy*, *10*(8), 1149.

Wolz, K. J., Branham, B. E., & DeLucia, E. H. (2018). Reduced nitrogen losses after conversion of row crop agriculture to alley cropping with mixed fruit and nut trees. *Agriculture, Ecosystems & Environment*, *258*, 172-181.

Wu, H., Cai, A., Xing, T., Huai, S., Zhu, P., Xu, M., & Lu, C. (2021). Fertilization enhances mineralization of soil carbon and nitrogen pools by regulating the bacterial community and biomass. *Journal of Soils and Sediments*, *21*, 1633-1643.

Zhang, H., Frey, M., Navizaga, C., Lenzo, C., Taborda, J., Taifan, W., Sadeghnejad, A., Sviklas, A.M., & Baltrusaitis, J. (2016). Dairy Wastewater for Production of Chelated Biodegradable Zn Micronutrient Fertilizers. *ACS Sustainable Chem. Eng., 4*: 1722−1727. DOI: 10.1021/acssuschemeng.5b01688

Zhang, S. L., Li, Z. J., Liu, J. M., Li, Q. H., Yang, X. Y. (2015). Long-term effects of straw and manure on crop micronutrient nutrition under a wheat-maize cropping system. *Journal of Plant Nutrition, 38*(5):742–753.

Zhang, J. B., Zhu, T. B., Cai, Z. C., Qin, S. W., & Müller, C. (2012). Effects of long-term repeated mineral and organic fertilizer applications on soil nitrogen transformations. *European Journal of Soil Science*, *63*(1), 75-85.

Zheng, H., Liu, W., Zheng, J., Luo, Y., Li, R., Wang, H., & Qi, H. (2018). Effect of long-term tillage on soil aggregates and aggregate-associated carbon in black soil of Northeast China. *PloS one*, *13*(6), e0199523. https://doi.org/10.1371/journal.pone.0199523

Zhong, W., Gu, T., Wang, W., Zhang, B., Lin, X., Huang, Q., & Shen, W. (2010). The effects of mineral fertilizer and organic manure on soil microbial community and diversity. *Plant and Soil*, *326*(1–2), 511–522. https://doi.org/10.1007/s11104-009-9988-y

Zhou, P., G. X. Pan, R. Spaccini, and A. Piccolo. 2010. Molecular changes in particulate organic matter (POM) in a typical Chinese paddy soil under different long-term fertilizer treatments. *European Journal of Soil Science*, 61: 231-242.