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Soil Management Strategies for Enhancing Soil Health and Productivity in California's Agricultural Systems: From Cover Crops to Vermicompost and Predictive Modeling

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## **Publication Date**

2024

Peer reviewed|Thesis/dissertation

Soil Management Strategies for Enhancing Soil Health and Productivity in California's Agricultural Systems: From Cover Crops to Vermicompost and Predictive Modeling

> by VERONICA SUAREZ ROMERO

### **DISSERTATION**

Submitted in partial satisfaction of the requirements for the degree of

## DOCTOR OF PHILOSOPHY

in

Agricultural and Environmental Chemistry

in the

## OFFICE OF GRADUATE STUDIES

of the

### UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

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> Committee in Charge 2024

## **Abstract**

This thesis explores different approaches to enhancing soil health and agricultural productivity

in California's Central Valley, a region facing significant challenges due to intensive farming

practices, soil degradation, and the need for sustainable management strategies. The document is organized into three key studies, each addressing different aspects of soil management and

their implications for short-term sustainability.

## **Chapter 1**

## **Short-term Approaches: Balancing Cover Crop Practices for Soil Health and Carbon Sequestration in the Central Valley in California**

-in collaboration with Geoffrey Koch

## **Chapter 2**

## **Replacing Synthetic Nitrogen Fertilizer with Vermifiltration By-Products: Effects on Soil and Walnut Tree Health**

## **Chapter 3**

## **Evaluating the Predictive Capability of Saturated Paste for Soil Bulk Density in Annual Cropping Systems in California**

## **Acknowledgments:**

I want to express my deepest gratitude to God for the strength and wisdom provided throughout this journey. To my family, whose unconditional support and encouragement have been my anchor, thank you for believing in me. I am immensely grateful to my professors, whose guidance and expertise have been invaluable in shaping this research and have inspired me. Special thanks to my advisor, Dr. William Horwath, for his endless patience and insight. To my colleagues, thank you for your support and shared experiences that have made this journey enjoyable and enriching. This dissertation would not have been possible without each of you.

## **Chapter 1 - Short-term Approaches: Balancing Conservation Agriculture Practices for Soil Health and Carbon Sequestration**

#### **1- Introduction**

The need for productive agricultural soils has increased dramatically over the last 50 years (Horwath and Kuzyakov, 2018), yet losses of topsoil and soil function due to intensive farming still amount to over \$8 billion a year (Sartori et al., 2019). Soils in Mediterranean or semi-arid regions face a high risk of degradation and are simultaneously under significant pressure to maintain high agricultural productivity. Factors like increasing population, rapid land-use changes, severe drought, and increased soil salinity exacerbate the susceptibility of these regions to soil degradation (Bidalia et al., 2019; Ferreira et al., 2021; Jacobsen et al., 2012). To address this issue, it is essential to implement soil conservation practices that can potentially reverse degradation and enhance soil health (Moebius-Clune et al., 2018; Mohawesh et al., 2015).

Soil health refers to the ability of soil to function as a living system, supporting biological productivity, maintaining environmental quality, and promoting health for plants, animals, and humans (Doran & Zeiss, 2000). Soil is a biologically active entity that sustains various ecosystem services, including net primary productivity, food and nutritional security, biodiversity, water purification, renewability, carbon sequestration, air quality, atmospheric chemistry, and nutrient cycling (Cogger & Brown, 2016; Kopittke et al., 2019). All these ecosystem services contribute to human well-being and nature conservancy (Lal, 2016). Soil health is a critical factor in climate change mitigation, as soils act as a significant reservoir for sequestering carbon and reducing greenhouse gas emissions in the atmosphere (Radulov et al., 2023). Improving soil health can enhance soil functions and resilience, like biological productivity and maintaining plant health, which is a major function of agroecosystems and is important to mitigate climate change (Kopittke et al., 2019; Lal et al., 2011; Raghavendra et al., 2020).

 Despite the increasing scientific evidence, there are still ongoing discussions about what constitutes a healthy soil and which indicators are the most useful for measuring changes in soil health (Chambers et al., 2016; Karlen et al., 1997; Kravchenko & Robertson, 2011; Minasny et al., 2017; Morgan et al., 2020). Different chemical, physical, and biological indicators are used to

assess soil health. These measurable attributes relate to functional soil processes and evaluate soil health on a defined timescale (Raghavendra et al., 2020). The North American Project to Evaluate Soil Health Measurements *(NAPESHM),* led by the Soil Health Institute, aimed to define the minimum appropriate parameters necessary to measure soil health (Norris et al., 2020). This project evaluated more than 20 indicators using data from more than 120 different sites across the United States, Canada, and Mexico and determined which indicators respond to management and, therefore, are the most useful in measuring soil health. However, there is still a gap in developing reliable soil health indicators, especially tailored for arid and semi-arid regions (Omer et al., 2018). By having indicators sensitive to the specific conditions of these regions, it becomes possible to assess soil health and implement targeted management accurately.

 Measuring indicators such as soil carbon, nitrogen, POXC, pH, wet aggregate stability, and water infiltration is crucial for accurately assessing soil health. These indicators offer valuable insights into the soil's nutrient content, structure, and ability to support plant growth. For example, soil carbon and nitrogen levels are essential for understanding nutrient availability and carbon sequestration (Tiefenbacher et al., 2021; Wei et al., 2024), and serve as food sources for the soil microbial community (Lal, 2016). Soil pH and EC play a significant role in influencing soil nutrient availability and plant uptake while establishing a threshold for plant and microbial growth (Allen et al., 2011). Additionally, wet aggregate stability and water infiltration rates are key indicators of soil structure and water retention capacity and are important for assessing how soils can adapt to a changing climate with severe rainfalls or drought (Raghavendra et al., 2020).

 Cover cropping is a widely known conservation practice to promote soil health (Crystal-Ornelas et al., 2021; Kim et al., 2020; Nunes et al., 2020; USDA, 2016a). Horwath and Kuzyakov (2018) found that implementing cover cropping can optimistically expect to maintain or increase soil carbon while increasing soil health. Cover crops enhance soil structure by increasing soil organic carbon, reducing bulk density, and promoting the formation of macropores, which are essential for water infiltration and retention (Haruna et al., 2020). Cover crops also mitigate soil erosion by providing ground cover that reduces the impact of raindrops and surface runoff (Folorunso et al., 1992; Ruan et al., 2001). Including cover crops in agricultural systems has shown to significantly increase water infiltration rates compared to soils without cover crops, thereby

improving soil moisture retention and reducing the risk of drought stress (Haruna et al., 2020). Additionally, cover crops enhance soil microbial activity and biodiversity, which further improves soil fertility and structure (Rankoth et al., 2019). By integrating cover crops into crop rotations, reliance on synthetic fertilizers can be reduced, as leguminous cover crops can fix atmospheric nitrogen and reduce nitrogen inputs (Kladivko et al., 2014; Sadra et al., 2024). Moreover, cover crops help reduce soil compaction, improve aeration, and facilitate root penetration, which is crucial for optimal plant growth (Cercioglu et al., 2018).

 The variability in soil health benefits derived from cover crops in arid or semi-arid regions is influenced by climate, soil type, cover crop species, management practices, and duration of cover crop use. The semi-arid climate often limits the growth of cover crops due to insufficient moisture, which can lead to competition for water with subsequent cash crops (Reese et al., 2014). Some studies have shown that cover crops can reduce soil water availability, thereby potentially decreasing yields of subsequent crops if not managed properly (Liebig et al., 2015; Nielsen et al., 2015). The timing of cover crop termination is also critical; if cover crops are not terminated early enough, they may compete for moisture, which is a limited resource in these regions(Lee & McCann, 2019). Conversely, the mulch created by cover crops can help reduce evaporation, thus conserving soil moisture (Kaye & Quemada, 2017). Soil type and initial soil organic carbon (SOC) levels contribute to the variability in soil health benefits from cover crop use. Research indicates that the response of SOC to cover cropping is influenced by the initial SOC content and soil texture (Blanco-Canqui, 2022; Chu et al., 2017). The choice of cover crop species or mixtures also significantly impacts the outcomes. Different species exhibit varying abilities to enhance soil health through biomass production, nutrient cycling, and microbial community support (Wittwer et al., 2017).

 Considering that the positive impacts of cover crops on carbon stocks can be variable and limited in more arid climates and that systems in California are not only arid but intensively managed and irrigated, and that data on the impacts of these practices in the west are very limited (Davidson and Janssens, 2006; Liptzin et al., 2022.; Ogle et al., 2012) there is a need to assess the impact of cover crops in Californian systems.

Most of the research regarding cover cropping and soil carbon comes from east of the Rocky Mountains in climates with warm, wet summers and long, frozen winters (Norris et al., 2020; Powlson et al., 2014). In contrast, California's climate is characterized by short, cool, and wet winters and long, hot growing seasons under irrigation. This big difference in climate promotes microbial decomposition and carbon respiration for much more of the year than in other places, creating differences in the impact of sustainable agricultural practices. This lack of adequate, reproducible data for semi-arid regions and whether these healthy soil practices will have the same positive effects as in other climates is currently an active area of research.

Investigating the impact of cover crops on soil health in California is imperative due to the significant discrepancies in soil and climatic conditions between California and the Midwest, where most existing cover crop research has been conducted. The climatic and soil differences necessitate region-specific research to determine the efficacy and benefits of cover crops in California's diverse agricultural systems. Additionally, the current data for California primarily derive from long-term managed cover crop sites at experimental stations(Brennan, 2020; Brennan & Acosta-Martinez, 2019; Brennan & Boyd, 2012; Brennan & Smith, 2017; Folorunso et al., 1992; Gomes et al., 2023; Maltais-Landry et al., 2014, 2015, 2015; Mitchell et al., 2015, 2017; Schmidt et al., 2018; White, Brennan, & Cavigelli, 2020; White, Brennan, Cavigelli, et al., 2020; White et al., 2022), which may not accurately reflect the conditions encountered on commercial farms. Conducting on-farm trials allows for incorporating local farmer preferences and management practices, yielding results that are more relevant and applicable to the region(Wood & Bowman, 2021). Our approach bridges the gap between controlled research environments and the practical realities of farming, thereby providing insights into more effective and regionally adapted cover crop strategies to enhance soil health and achieve agronomic and environmental benefits.

Moreover, data on conservation agriculture practices primarily focuses on the top 30 cm of soil profiles. In the last decade, several studies have established the importance of considering more of the soil profile before broad conclusions are made (Balesdent et al., 2018; Harrison et al., 2011; Tautges et al., 2019). For example, Tautges et al. (2019) found that when only considering the first 30 cm of the soil profile, soil carbon concentrations appear to increase in

response to cover crops and compost applications. However, when considering the whole profile to 1-2 meters, this increase disappears, and cover-cropped treatments lose carbon in the subsurface.

In the present study, we aimed to fill the knowledge gaps identified in previous research by investigating the impact of cover cropping on soil organic carbon stocks and other soil health indicators across varying depths in short-term managed sites within arid to semi-arid Mediterranean conditions. We hypothesize that cover cropping will enhance soil health indicators, specifically, we expect cover cropping to increase soil carbon and nitrogen stocks, particularly in the upper soil layers. We anticipate that POXC will increase in cover-cropped plots, due to fresh plant residue incorporation, while soil pH will decrease due to organic matter decomposition and root exudation of organic acids. Additionally, wet aggregate stability and water infiltration rate will improve with cover cropping due to increased root biomass and organic matter inputs. Enhanced soil structure, along with increased water storage and movement through the soil profile with cover crops, is expected to lower soil electrical conductivity by promoting salt leaching during irrigation.

### **2 - Material and Methods**

#### **2.1- Field trials**

In this study, we collected data from three on-farm field trials from 2018 to 2020, evaluating the short-term effects of crops on soil carbon and soil health representative areas of California's agricultural landscape. The trials were located along a gradient of decreasing precipitation and different soil types from Sutter County in the Sacramento Valley (CA, USA) to Fresno County in the southern San Joaquin Valley, representative of important agricultural areas of California. A summary of the sites studied is provided in **Table 1.**

#### **2.1.1 – Short-Term Legume Winter Cover Crop Management**

This trial was conducted in Sutter County (California, USA). The trial, started in 2018, with practice implementation in the winter. The experiment consisted of an RCBD with 3 treatments and 3 blocks. Vetch (*Vicia americana*) was planted as a winter cover crop at two rates (32 and 64 kg ha<sup>-1</sup> in the first year and 16 and 32 kg ha<sup>-1</sup> in the second year) and a control with no cover crop. These treatments were applied to 8-bed plots replicated three times. Soils are mapped as Shanghai, silt loam, clay substratum - 90 %, and similar soils (*fine-loamy, mixed, superactive, nonacid, thermic Aquic Xerofluvents*). Annual precipitation was reported to be 37 cm on average during the study year(*NOAA NCEI U.S.*, 07/24/2024)The previous crop history in this site was conventionally grown rice in 2015 and 2016, which was left fallow in 2017. Processing tomatoes were planted at the end of April 2019 and harvested in August 2019. As part of the grower practices, poultry manure compost was added to all the plots, including the control, in Fall 2018 at an approximate rate of 11.2 ton ha<sup>-1</sup>.

#### **2.1.2 - Short-Term Mixed Winter Cover Crop Management**

The field trial was located in Mendota, Fresno County (California, USA) and has been in cotton (*Gossypium barbadense or hirsutum*) production for 11 years (2011-2017), preceding that, the field was planted to alfalfa (*Medicago sativa)* for four years. The experiment consisted of a split-plot design with two treatments, including the control, and was established in 2018. The two treatments were cover crop (CC) and no cover crop or control (NO). In 2019, for the CC treatment, a combination of 95% small grain (usually Triticum aestivum or Triticosecale Wittm. Ex A. Camus) and 5% tillage radish (Raphanus raphanistrum) was planted by drilling. In 2020, a legume mix was applied and flown by plane. Treatments were applied and replicated three times across three blocks. The soil in two experimental blocks is classified as 85% *Elnido* series (*Coarseloamy, mixed, superactive, thermic Typic Endoaquolls).* The soil in the third block is classified as 85% *Palazzo series* (*Fine-loamy, mixed, superactive, thermic Fluvaquentic Endoaquolls)*.

All experimental plots received 4.48 t ha<sup>-1</sup> poultry manure compost in 2018 and 2019, and gypsum was applied in 2019 to address poor infiltration. The cover crop treatment was not irrigated in any year. The cotton crop was irrigated by a furrow-flooding once every seven days during the growing season.

Cover crops were terminated yearly by applying herbicide (glyphosate), flail-mowed, and incorporated by discing. After this, normal practices of rototilling and bed-formation preceded the planting of the cotton cash crop.

#### **2.1.3 – Short-Term Legume Summer Cover Crop Management**

The site was located in the Delta of the San Joaquin Valley, Merced County (California, USA). The short-term experiment started in 2018, with cover crop implementation in the summer of 2019 and 2020. Cowpea (*Vigna unguiculata* cv. 'Red Ripper) cover crop inoculated with Rhizobium was planted as a summer cover crop (rate 51 kg ha<sup>-1</sup> 2018; 56 kg ha<sup>-1</sup> 2019; 50 kg ha<sup>-</sup> <sup>1</sup>) and drill-seeded at 18-cm row spacing, compared to a control with no cover crop. Plot size was approximately 0.3 ha, with three replicates in a randomized complete block design. Soils are mapped as Rindge mucky silt loam, partially drained, 85 %, and similar soils (*Euic, thermic Typic Haplosaprists*). Irrigation was only applied to the cover crop plots to avoid volunteer weed growth in the control. In the first year, planting occurred after a flood/furrow pre-irrigation, and one additional irrigation was applied approximately one month later. Due to the slope of the field, however, water infiltration was uneven. In the second, planting occurred in dry soil, and the cover crop was irrigated with sprinklers. In 2019, 127 mm of irrigation was applied to the cover crop, using surface water with moderately low salinity (seasonal ECw of 0.5 dS/m).





## **2.2. – Soil and Cover Crop Biomass Sampling**

Soil sampling was carried out annually from 2018 to 2020 at all sites in the periods indicated in **Table 1**. To evaluate soil organic carbon, sampling was done to a depth of 90 cm in

2018 and 2020. In 2019, soil samples were collected to a depth of 30 cm. Sub-samples from each plot were collected using a soil auger, composited, homogenized in the field, and brought back to the lab for processing in sealable gallon plastic bags. Soils were then air-dried and sieved to <2mm, removing any visible plant residues. A subsample of unsieved soil was kept for aggregate stability analysis.

Soil Bulk density was determined by taking three 100  $\text{cm}^3$  soil cores from the middle of every depth at each plot. The soil cores were transported to the lab, oven-dried, and the mass was recorded.

Cover crop biomass was assessed before termination and incorporation of cover crops to the extent possible. Three one-meter squared (m<sup>2</sup>) sections of the cover crop were harvested by hand, cutting to the ground, drying, averaging dry biomass, and extrapolating results to the field level.

#### **2.3. - Soil Analysis**

Sieved samples were analyzed for soil pH, electrical conductivity (EC), and Permanganate oxidizable carbon (POXC). Briefly, Soil pH and EC were measured using 1:2 soil: DI water slurries, using a pH/EC probe (Mettler Toledo, Columbus, OH, United States) according to Allison & Richards (1954). POXC, used as a proxy for active carbon, was determined according to Weil et al.(2003) by oxidizing 2.5 g of air-dried soil with 20.0 mL of 0.02M potassium permanganate solution, shaking for 2 min, diluting a 0.5mL aliquot 100 times with DI water, and measuring the remaining permanganate concentration colorimetrically at 550nm (Shimadzu Scientific Instruments Inc. model UV-1280). Analytical replicates were accepted when the coefficient of variation (CV) was  $\leq$  20%.

 Pulverized soil samples were analyzed for total organic carbon and nitrogen by dry combustion (International, 1997) using an elemental analyzer (Costech Analytical Technologies Inc. model EAS32). The change in soil carbon and total nitrogen (N) stock was calculated as the difference between tons of total carbon per hectare in the soil at the beginning (year 1) and end (year 3) of each experiment for every site. Values were calculated by experimental unit (plot),

using bulk density and core length (15 cm) to convert from the percentage of total carbon to tons  $C$  ha<sup>-1</sup>.

Un-sieved samples were analyzed for aggregate stability using the Slake test kit (NRCS, 2001). Briefly, 16 soil aggregates (<2-5 mm) were separated manually from the 0-15 cm soil samples. Aggregates were placed in a mesh basket included in the kit, submerged in 2 cm of deionized water, and assessed to see how well aggregates withstand breakdown after 5 min of immersion and five dipping cycles. Each replicate was assigned a stability class according to the table provided in the kit. Stability class values were averaged for each treatment at each site.

Water infiltration measurements were collected for all sites and at each plot at the end of each experiment using a single-ring infiltrometer method (NRCS, 2001), except for the Sutter site, where data collection was not possible due to changes in land management.

### **2.4. Statistical Analysis**

Data were analyzed separately for each site, with data only being presented together to understand the broader patterns of cover crop implementation. Data was analyzed using RStudio (R Development Core Team, 2020). Linear models were built using the "lmer" function and used to performed ANOVA comparing treatment groups for each soil depth section separately. Cover crops were analyzed as a fixed factor and blocks as a random factor to account for random variation associated with spatial variability. For soil pH and EC, data collected over the studied period was analyzed with a linear model to each depth, and the variable year as a random factor to account for interannual environmental variability (precipitation and temperature). ANOVA results were analyzed for significant differences between treatment groups with  $\alpha$  = 0.05 (95% confidence interval). If treatments were significantly different according to these criteria, then a post hoc Tukey test was used to confirm the statistical significance between treatments.

### **3. Results and Discussion**

## **3.1 Effects of the Cover Crops on Soil Carbon Indicators**

### **Soil Total Carbon (SOC)**

Cover crops provide an additional source of aboveground and belowground crop residue carbon entering the soil, which can lead to enhanced soil carbon storage (Poeplau & Don, 2015). The impact of cover crops on soil carbon stocks varied with site and soil depth (**Figure 1**). In the **Delta**, using cover crops during the summer reduced soil carbon stocks, especially at 0-15 cm and 15-30 cm deep. Conversely, in **Mendota**, there was a decrease in cover crop plots carbon stocks at 0-15 cm, but increases were noted in the 30-60 cm and 60-90 cm depths. In contrast, **Sutter** showed no changes in carbon accumulation across all depths.



**Figure 1.** Change in carbon stocks after three years of cover crop use by depth at three different cover crop trials in Central Valley, California. Error bars represent the standard error of the mean. Significant differences between treatments within depth intervals at 95% confidence are marked with \*, and cover crop effect p-values obtained through ANOVA are presented.

 At the **Delta** Site (**Figure 1A**), cover crop (CC) accumulated significantly less (p<0.001) carbon than the fallow control. The cover crop plots accumulated an average of 2.06 Mg C/ha at 0-15 cm, which was significantly ( $p < 0.001$ ) lower than the 5.24 Mg C/ha accumulated by the fallow control after three years of management. Additionally, at 15-30 cm, the fallow control accumulated 85% more carbon ( $p < 0.001$ ) than the cover crop, with a mean value of 11.62 Mg C/ha and 1.69 Mg C/ha, respectively. There was no significant difference ( $p = 0.87$ ) in carbon stocks for the 30-60 cm depth, with the cover crop treatment accumulating 2.2 Mg C/ha and the fallow control accumulating 3.07 Mg C/ha. At 60-90 cm, the cover crop accumulated 3.84 Mg C/ha, and the fallow control accumulated 5.79 Mg C/ha, with no significant difference ( $p = 0.53$ ) between them. These unexpected findings contradict our initial hypothesis that soil carbon stocks would increase through carbon inputs via cover crop biomass. The use of cover crops during the summer, traditionally reserved for cash crop cultivation, remains relatively underexplored in this region. At the Delta site, alfalfa is the primary crop grown throughout the year, leaving the soil fallow during the summer months. Implementing a summer cover crop aimed to mitigate wind erosion and soil degradation—two significant environmental concerns within the Sacramento-San Joaquin Delta region (Deverel et al., 2020). However, the need for irrigation to support cover crop establishment during the summer may have inadvertently caused carbon loss by increasing soil respiration rate while not providing enough carbon inputs to increase carbon stocks. Additionally, increased soil disturbance through tillage and cover crop incorporation disrupts the aggregates in the soil surface, and this could have exposed carbon occluded in the soil aggregates, making it prone to microbial degradation (Poeplau & Don, 2015; Six et al., 2000).

At the **Sutter** site, planting a winter CC over three years did not increase soil C stocks compared to the control. We observed a trend of increased average carbon stocks with cover cropping in the 0-15 cm depth section, although the differences were not statistically significant (p=0.07). Specifically, the carbon accumulation was 8.68 Mg C/ha for the Control, 10.12 Mg C/ha for Low CC, and 11.42 Mg C/ha for High CC, indicating a trend of higher carbon stocks with increased cover cropping intensity, but without significant differences between the treatments.

In the 15-30 cm depth section, carbon accumulation was recorded at 6.18 tons/ha for Control, 6.53 tons/ha for Low CC, and 6.81 tons/ha for High CC. Despite slightly higher values with increased cover cropping intensity, these differences were not statistically significant (p=0.98).

For the 30-60 cm depth, carbon stocks were 4.42 tons/ha for Control, 3.80 tons/ha for Low CC, and 3.70 tons/ha for High CC. Again, the variations between treatments did not show significant differences (p=0.17). At the 60-90 cm depth, the trend continued with carbon stocks measuring 4.47 tons/ha for Control, 3.38 tons/ha for Low CC, and 2.94 tons/ha for High CC. Similar to the other depth sections, these results also indicated no significant differences between the treatments (p=0.1666). The addition of compost to all plots, including the control, and volunteer weeds in the control likely hid potential CC effects in SOC. This increase in SOC because of composted poultry manure inputs in combination with winter CC has been observed in similar studies in California for tomato-maize rotation (Tautges et al., 2019). Our findings corroborate the observations from a model generated by Bierer et al. (2021), which indicated that manure applications have a higher SOC increase in short-term management (three years) than cover crops. In addition, similar to our findings, Thapa et al. (2021) found that after five years of using different winter cover crop types in a semi-arid region, the SOC was not affected compared to the control, but potential mineralizable carbon and POXC increased, suggesting that more years are needed to measure significant changes in SOC. Bierer et al. (2021) modeled the impact of cover crops and manure in semi-arid regions, showing that cover crops could increase SOC accrual rates by 0.05 to 0.27 Mg ha<sup>-1</sup> yr<sup>-1</sup> at 0-15 cm after 50 years, depending on biomass input and microbial activity. While cover crops moderately increase SOC in the short term, cover crops are essential for long-term soil health and fertility by providing carbon and nitrogen to sustain the soil microbial population and stabilize carbon. Similarly, Schmidt et al.,(2018) found that long-term cover cropping in the Mediterranean climate leads to changes in soil microbial communities, promoting greater resource diversity and nutrient availability due to increased organic carbon inputs from cover crop residues and shorter fallow periods.

At the **Mendota** site, cover crop had different impacts on carbon stocks depending on the depth (**Figure 1 C**). At 0-15 cm, the cover crop lost an average of 3.64 kg ha<sup>-1</sup> more carbon than the control (fallow). The decrease at 0-15 cm can be explained by the unsuccessful cover crop

establishment in the first and third years of the study period ( $\sim$ 1.24 Mg ha<sup>-1</sup> in 2018,  $\sim$ 7.41 Mg ha<sup>-</sup> <sup>1</sup> in 2019, and ~0.25 Mg ha<sup>-1</sup> in 2020) and the addition of compost (~ 4.5 ton ha<sup>-1</sup>) was not adequate to increase or even maintain carbon stocks in this intensively managed cotton system. However, the cover crop increased carbon stocks by 36% and 60% compared to the fallow treatment at the subsequent depths of 30-60 cm and 60-90 cm, respectively. This finding highlights the importance of considering the whole profile to at least 1-meter depth, as surface conditions are much more variable due to higher disturbance. Notably, this site received flood irrigation, and carbon observed at depth could have been deposited and translocated in subsequent years. Movement of dissolved organic carbon has been demonstrated through wetdry cycles (including irrigation) in other Californian systems (Lundquist et al., 1999). A change in texture at a depth of 30 cm (increase in depositional clay) was observed in all the plots at this site. This change can explain carbon accumulation at 30 cm, as it is just below the layer regularly disturbed with tillage. The increase in clay at this depth was noticeable as the surface is notably coarse-textured. Other studies have shown that higher clay content positively affects carbon accumulation due to carbon sorption on clay particles (Schweizer et al., 2021). In addition, the Mendota site received a cover crop mixture of legumes and grasses, which are deep-rooted and allow for carbon to percolate through the soil profile. Another possible mechanism to explain carbon accumulation at 30-90 cm is that cover crops, especially grasses, can transport carbon deeper into the soil profile through rhizodeposition (the release of organic compounds by roots) (Kell, 2011). This increases carbon inputs at greater depths where it is less susceptible to rapid decomposition due to lower microbial activity and physical isolation from soil disturbances (Button et al., 2022). There is a potential to increase carbon stocks by using cover crops at this site if carbon continues to accumulate at 30-90 cm depth. Other researchers have reported similar effects in semi-arid environments. For instance, Hux et al., 2023 demonstrated that a singlespecies wheat cover crop in a no-till system produced higher carbon mineralization rates than nocover crop scenarios.

Long-term cover crop experiments in California have shown that cover crops positively impact soil organic carbon, particularly in surface layers (0-30 cm)(Mitchell et al., 2017), and contribute significantly to labile carbon, enhancing soil health (White, Brennan, Cavigelli, et al.,

2020). However, these studies combined cover crops with no-tillage practices or compost additions, reporting a cofounded effect of these practices. In addition, most of the outcomes are reported for the topsoil at 0-30 cm. The effect of cover crops may vary with soil depth. For example, Tautges et al., 2019 found that SOC stocks increased by 3.5% (1.44 Mg C/ha) in the 0– 30 cm layer, but decreased by 10.8% (14.86 Mg C/ha) in the 30–200 cm layer, resulting in overall losses of 13.4 Mg C/ha after 19 years of cover crop use. The results from our short-term experiments highlight that soil depth and texture play an important role in the effect of cover crops on soil organic carbon accumulation.



### **Permanganate Oxidizable Carbon (POXC)**

**Figure 2.** Soil POXC concentrations at different depths for three cover crop trials in the Central Valley in California, data for 2020. Cover crop effect p-values from ANOVA analysis at each depth are reported.

POXC is thought of as a proxy for the labile fraction that is supposed to be responsive to short-term management (Culman et al., 2012; Hurisso et al., 2016; Wade et al., 2020). The increase in POXC can predict carbon accrual (Culman et al., 2012). We did not observe an impact of cover crops on POXC at any depth in any of our study sites (**Figure 2**).

At the **Delta** site (**Figure 2a**), POXC concentrations were not significantly different between the cover crops (Low CC and High CC) and the control (fallow), with mean POXC values of 850 mg C/Kg soil at 0-15cm (p = 0.82), 876 mg C/Kg soil at 15-30 cm (p = 0.73), 525 mg C/Kg soil at 30-60 cm ( $p = 0.73$ ), and 345 mg C/Kg soil at 60-90 cm ( $p = 0.55$ ). The lack of statistical differentiation

may be attributed to the high temperature and irrigated conditions during summer. It is possible that the cover crop biomass inputs made were rapidly degraded and respired away as  $CO<sub>2</sub>$ . Labile soil carbon substrates are smaller, more spatially accessible, and have lower molecular weight, therefore, they have a higher capacity for microbial assimilation (Erktan et al., 2020; Kallenbach et al., 2015). In addition, their high solubility and the presence of polar functional groups facilitate their sorption interactions with mineral surfaces, increasing their protection and persistence through stabilization within the soil matrix(Cotrufo et al., 2013). Therefore, there is a balance between the different stabilization pathways, including the microbial metabolic processing of these substrates, that results in partial loss of SOC through respiration and the flow of carbon through the soil profile as microbial-derived compounds or direct protection through sorption with soil minerals (Moukanni et al., 2022).

In the **Mendota** site, cover crops did not impact soil POXC. Average POXC values for this site were 445 mg C/Kg soil at 0-15 cm (p = 0.42), 404 mg C/Kg soil at 15-30 cm (p = 0.57), 366 mg C/Kg soil at 30-60 cm( $p = 0.57$ ), and 215 mg C/Kg soil at 60-90 cm( $p = 0.90$ ). POXC decreased with depth at most of our sites, a trend that others have reported (Culman et al., 2012; Wade et al., 2020), which usually correlates with the total carbon content of each depth. However, the **Mendota** site (**Figure 2b**) had an interesting pattern, where POXC didn't decrease with depth at the first 60 cm. The cover crop plots at this site tended to increase POXC with depth. This trend could be explained by a subsurface layer with higher clay content at 30-60 cm. The accumulation of oxidizable carbon could have been produced by deposited root exudates that contain labile sugars and organic acids from the cover crop roots(Panchal, 2022). Carbon accumulation at deeper layers can be a promising route for stable carbon accumulation due to higher protection and reduced soil disturbances (Wade et al., 2020). The different pattern of labile carbon allocation by depth between the cover crop and control at this site agrees with other studies that found that cover crops can alter the formation and size distribution of soil pores, allowing carbon to move down the soil profile with water (Panchal et al., 2022).

 At the **Sutter** site (**Figure 2c**), we noticed a similar trend compared to the other sites, where the cover crops had no impact on POXC at any depth. The average POXC values at each depth were 507 mg C/Kg soil at 0-15 cm (p = 0.71), 354 mg C/Kg soil at 15-30 cm(p = 0.60), 237 mg C/Kg

soil ( $p = 0.62$ ), and 283 mg C/Kg soil at 60-90 cm( $p = 0.58$ ). At this site, the cover crop effect could be hidden by the previous addition of compost to all plots and the presence of volunteer weeds in the control, that contribute labile biomass inputs. There is also the possibility that POXC is not capable of detecting the differences induced by cover crop management. Hurrisso et al. (2016) found that POXC was more sensitive to compost use, while C mineralization measurements were more sensitive to cover crops. Our findings compare with previous research in the same region, where the combination of winter cover crops and compost didn't impact POXC in the first years of the study but showed increased POXC after five years(White, Brennan, Cavigelli, et al., 2020).



#### **3.2 Effects of the Cover Crops on Soil Nitrogen**



Cover crops had a variable impact on soil nitrogen(**Figure 3**) depending on the site and soil depth, with the most pronounced effect observed in the 0-15 cm soil depth at the Delta site. At the **Delta** site (**Figure 3A**), the cowpea legume cover crop significantly decreased total nitrogen in the 0-15 cm depth ( $p < 0.001$ ) compared to the control. However, no significant effects were observed at the deeper depths of 15-30 cm (p = 0.34), 30-60 cm (p = 0.74), and 60-90 cm (p =

0.48). This observation contradicted our hypothesis that legume cover crops would increase soil total nitrogen through nitrogen fixation from the atmosphere and contributions from biomass inputs. Even though nitrogen fixation could have increased at this site, higher soil respiration during the summer could have reduced the carbon and nitrogen stocks at the cover crop treatment, compared with the control, which was fallow and dry over the summer. In summer, the active growth of crops can lead to significant nitrogen uptake from the upper soil, depleting the soil nitrogen pool available at this depth. A possible mechanism to explain our observation is that stimulation of microbial activity due to fast cover crop growth rates under irrigation during summer leads to the decomposition of soil organic matter and faster nitrogen mineralization (Moukanni et al., 2022). Mineral N could have been lost through volatilization due to high temperatures and/or leaching caused by irrigation (Quemada et al., 2013), resulting in a net decrease in soil total nitrogen in the irrigated cover crop plots.

At the **Sutter** site (**Figure 3B**), there was no significant change in total nitrogen in the 0-15 cm (p = 0.33), 15-30 cm (p = 0.72), 30-60 cm (p= 0.55) and 60-90 cm (p = 0.15) depths between different rates of cover crop and control treatments. At the **Mendota** site (**Figure 3C**), the cover crops did not significantly affect total nitrogen at the 0-15 cm depth (p = 0.77), 15-30 cm depth  $(p = 0.92)$ , and 30-60 cm depth  $(p = 0.25)$ . However, the 60-90 cm depth showed a trend toward significance ( $p = 0.07$ ), indicating a potential effect of cover crops on total nitrogen at this deeper layer. This finding agrees with work at Russel Ranch (Davis, CA), which showed that cover cropping increased carbon and nitrogen transport to the subsoil (Rath, 2022). Cover crops uptake residual available nitrogen, and this mechanism has been used to capture and prevent nitrogen leaching (Abdalla et al., 2019; Quemada et al., 2013).Our results suggest that using a cover crop mix influences nitrogen distribution deeper in the soil profile. The interaction between the shallowrooted legumes and deep-rooted grasses might create a nitrogen gradient within the soil profile. The legumes contribute nitrogen to the upper layers by fixing atmospheric nitrogen through symbiosis with rhizobia(Doane et al., 2009), while the deep-rooted grasses create preferential soil paths(Moukanni et al., 2022) and translocate nitrogen(Perkus et al., 2022), allowing it to mobilize deeper into the soil. Although there is a feedback mechanism when intercropping legumes with grasses, which can modulate the nitrogen fixation rate of legumes(Blesh, 2019). Grasses compete with legumes for soil nitrogen, potentially enhancing nitrogen fixation by legumes but also limiting their biomass(Perkus et al., 2022).

The failure of legume cover crops to significantly increase soil total nitrogen in our study can be attributed to several interrelated factors, including competition with other plants, environmental conditions, and interaction with other management practices(Franco et al., 2021; Veloso et al., 2018). The competition with other cover crops that may outcompete legumes for resources limits the nitrogen-fixing effect of legumes(Perkus et al., 2022). For example, at the **Delta** site, cowpea was the only cover crop seed planted; however, the count stand was a mix of cowpea, volunteer wheat/triticale, and weeds, with cowpea being on average 16% of the biomass stand counts, and the rest consisting of weeds and volunteer small grains. This competition can limit legume growth, limiting nitrogen fixation, and subsequent nitrogen inputs to the soil.

Another factor to consider is that nitrogen fixation by legumes is significantly influenced by the nitrogen availability in the soil. It has been observed that when there is abundant nitrogen in the soil, legumes may rely less on nitrogen fixation(Blesh, 2019). This is because the energy cost of obtaining nitrogen through a symbiotic relationship with rhizobia is higher than utilizing the nitrogen already present in the soil(Schipanski et al., 2010), which in our scenario could have been supplied by adding poultry compost.

#### **3.3 – Effects of the Cover Crops on Soil pH and EC**

#### **Soil pH**

Soil pH was unaffected by the cover crops in our study sites, as presented in **Figure 4**. There was a trend of increased soil pH by summer cover crops at the **Delta** site in the 0-15 cm and 15-30 cm. Winter cover crops did not affect soil pH at the **Mendota** and **Sutter**.



**Figure 4.** Soil pH *values* at different depths for three cover crop trials in the Central Valley, in California, data for 2018- 2020. The p-values from the cover crop effect were obtained through ANOVA analysis at each depth for the effect of cover crops across years.

 At the **Delta** site, soil pH averaged 6.5 at 0-15 cm (p=0.063), 6.4 at 15-30 cm (p=0.064), 6.5 at 30-60 cm (p=0.72), and 6.8 at 60-90 cm (p=0.47), with no significant differences between CC and fallow control treatments at any depth, although there is a trend of increased soil pH in the CC at 0-30 cm depth. The increase in pH can be attributed to several factors, including irrigation, the effect of root exudates, and enhanced cover crop decomposition. Irrigation can increase soil pH due to the presence of bicarbonates in the water, which act as a buffer and raise the pH level of the soil(Gardner, 2004). Cover crop root exudates that contain a diverse array of compounds, including organic acids, which can directly influence soil pH by either acidifying or alkalizing the surrounding soil (Ma et al., 2022; Seitz et al., 2023). For example, the release of carboxylic acids, such as citric and malic acid, can help to solubilize nutrients and enhance their availability, while also potentially increasing soil pH by neutralizing acidity (Balota & Chaves, 2011), this effect has been observed especially in low-pH soils (Heuermann et al., 2023). It can also be due to the decomposition of legume cover crop residue after incorporation in the soil, where the organic nitrogen is mineralized into the soil after decomposition, and processes like ammonification can increase the pH of the soil(Li et al., 2005). This has been reported, especially for legume cover

crops, where the initial soil pH affects the balance between the ammonification and nitrification processes of the cover crop residue decomposition (Vanzolini et al., 2017). Similar results were observed by Gao et al.(2022), who found that single-species and multi-species cover crops increased soil pH compared to a fallow control in an arid region in China.

The **Mendota** and **Sutter** sites maintained a similar pH to the fallow control at different depths. For Soil pH averaged 6.5 at both 0-15 cm (p=0.74) and 15-30 cm (p=0.44), 6.8 at 30-60 cm (p=0.76), and 6.9 at 60-90 cm (p=0.18), with no significant differences between the cover crop and fallow control treatments at any depth. At the **Sutter** site, soil pH averaged 6.6 at 0-15 cm (p=0.22), 6.5 at 15-30 cm (p=0.56), 6.6 at 30-60 cm (p=0.21), and 6.8 at 60-90 cm (p=0.18), with no significant differences between the cover crops and the fallow treatments at any depth. The effect on soil pH at these two sites can be influenced by other management practices like the addition of poultry litter compost. Poultry litter compost has been reported to have an alkaline pH around 9 (Kajiya et al., 2015). Other researchers have found increases in soil pH after consecutive annual additions of manure compost (Chen et al., 2022). Similarly, an experiment conducted by Khan et al. (2021) that evaluated the influence of cover crop residues and poultry litter compost found that, after one year of incorporation, soil pH was unaffected by most cover crop residues compared to the control.

#### **Soil Electrical conductivity (EC)**

Cover crops have been shown to have significant impacts on soil salinity, particularly in arid and semi-arid regions where salinity poses a major challenge to agricultural productivity (Gabriel et al., 2012; Tarolli et al., 2024).Low precipitation and high evapotranspiration contribute to soil salinization in arid environments, which adversely affects crop yields (Glick et al., 2007). Electrical conductivity (EC) was used to measure soil salinity in our study and was impacted differently across sites. At the **Delta** site, we observed that the cover crops generally decreased soil EC at all depths; however, cover crops did not affect soil EC at the **Mendota** and **Sutter** sites.

At the **Delta** site(**Figure 5A)**, we observed decreased EC for the CC plots compared to the fallow control at all depths. Soil EC at 0-15 cm had an average EC value of 0.4 dS/m for CC and 0.7 dS/m for the fallow control (p< 0.001). At 15-30 cm, significant differences (p = 0.03) showed EC values for the fallow control treatment with 1.0 dS/m and CC treatment with 0.8 dS/m. Significant differences in soil EC were observed at 30-60 cm ( $p = 0.007$ ) and 60-90 cm ( $p = 0.04$ ). The fallow control showed higher EC values of 2.5 dS/m and 3.03 dS/m compared to the CC plots, which had EC values of 1.3 dS/m and 2.0 dS/m at depths of 30-60 cm and 60-90 cm, respectively. The cover crop treatment had a lower EC due to the irrigation necessary to grow the cover crop compared to the dry fallow control. Although this effect may not be directly associated with cover crop growth, it represents an added benefit of this management practice at the Delta site. Soil salinization is a pressing issue in the San Joaquin Valley, primarily driven by factors such as irrigation practices, shallow water tables, and the natural saline conditions of the alluvial soils in the region(Corwin, 2021; Marino et al., 2019; Singh et al., 2020). The adoption of summer cover crops that require irrigation during the summer at this region could aid in reducing soil salinization, by maintaining soil cover and regulating moisture, which minimizes salt accumulation through evaporation(Dasgupta et al., 2023; Qi et al., 2023).

Soil EC at **Mendota** maintained relatively stable EC values across the years, with no impact from cover crops. Soil EC across all plots averaged 1.4 dS/m at 0-15 cm (p=0.46), 1.5 dS/m (p=0.12), 0.6 dS/m at 15-30 cm (p=0.85), 0.6 dS/m at 30-60 cm (p= 0.85) and 0.8 dS/m at 60-90 cm (p= 0.13). Similar findings were obtained at the Sutter site, where CC did not impact soil EC compared to the fallow control. Soil EC averaged 1.2 dS/m at 0-15 cm (p=0.11), 0.9 dS/m at 15- 30 cm (p=0.39), 0.8 dS/m at 30-60 cm (p= 0.29) and 0.8 dS/m at 60-90 cm (p= 0.16). The **Mendota** and **Sutter** sites were probably controlled by the poultry compost applied to all plots, including the control. Although we did not observe any significant effects of cover crops compared to the control at our study sites, compost quality and source are important factors to consider, especially in soils with existing salinity issues, as large additions of compost with high EC could result in elevated soil EC (Gondek et al., 2020) that would affect crop establishment and yield (Jamil et al., 2006) if not managed with adequate leaching irrigation to remove soluble salts.



**Figure 5.** Soil EC measurements at different depths for three cover crop trials in the Central Valley in California, data for 2018-2020. Cover crop effect p-values from ANOVA analysis at each depth for the effect of cover crops across years are reported.

### **3.4 – Effects of the Cover Crops on Wet Aggregate Stability and Water Infiltration**

### **Wet Aggregate Stability**

Cover crops did not impact wet aggregate stability (**Figure 6**), measured by the Slakes test, at any site. Moreover, at the **Delta** site, the control treatment (NO) showed a trend (p = 0.08) of higher wet aggregate stability scores than the cover crop treatment. At this site the cover crops averaged a stability score of 2.5, while the fallow control had an average score of 3.5, showing increased water stability. This suggests that operations like cover crop incorporation and irrigation, necessary to grow cover crops during the summer at this site, may cause higher disruption of the soil aggregates, further enhancing carbon loss, as was evidenced by the reduction in carbon stocks at this site. At the **Mendota** site, both treatments had an average wet aggregate stability score of 2; with no significant difference( $p = 0.75$ ) between the cover crop

treatment and the control. At the **Sutter** site, the cover crop treatments showed slightly higher wet aggregate stability scores: 2.6 for Low CC and 2.8 for High CC, compared to 2.4 for the fallow control. However, this difference was not statistically significant ( $p = 0.14$ ). These results suggest that the effect of cover crops on soil aggregate stability may vary by site, with no consistent pattern across the locations studied. These results contradict our initial hypothesis, where we expected that the effect of cover crop active roots would increase wet aggregate stability in the cover crop treatments, improving soil structure. Similar to our observations, a study conducted in a semiarid vineyard, evaluated different cover crop management systems on soil properties, including aggregate stability(Peregrina et al., 2014). Their findings showed that planting a cover crop had reduced aggregate stability compared to resident vegetation due to tillage during resowing. Contrary to our results, Mitchell et al., 2017 reported increased wet aggregate stability 19 years after the adoption of cover crops in a long-term study at Five Points in California. This coincides with a meta-analysis evaluating the global effects of cover crops on wet aggregate stability, which found that, in general, 75.4% of the reviewed plots showed an increase in WAS when cover crops were used compared to control treatments without cover crops (Hao et al., 2023).

The data suggest that sampling time and the use of more specific indexes of aggregate stability, such as mean weight diameter (MWD) and slow wetting (SW) tests, may capture the effects of cover crops more effectively than the traditional slake test used in our study(Dai et al., 2024; Hao et al., 2023). Cover crops can increase soil wet aggregate stability through various mechanisms. Root exudates, high in carbohydrates, work as a binding agent (Odesa, 1979) that holds soil particles together. In addition, root exudates promote microbial activity around the roots, especially fungal activity, which generates extracellular polymers that bind soil particles (Rillig & Mummey, 2006). Another mechanism that can increase aggregate stability is water uptake in the root zone, which causes localized wet-dry cycles that help stabilize the aggregates formed by the expansion and shrinking of clay minerals(Six et al., 2002). The formed soil aggregates protect carbon (Plaza-Bonilla et al., 2013) and can increase carbon sequestration through occlusion and protection from microbial access (Blanco-Canqui and Lal, 2010). The factors that determine how effective cover crops are in increasing aggregate stability are the number of cycles of cover

cropping, cover crop species, the number of aggregates, their size, clay content, and soil mineralogy. (Blanco-Canqui and Lal, 2010; Cotrufo et al., 2019; Jastrow et al., 2007; Plaza-Bonilla et al., 2013; Sundermeier et al., 2011).



Wet Agregate Stability Scores

1. 50% of structural integrity lost (melts) within 5 seconds of immersion in water AND < 10% remains after 5 dipping cycles, OR soil too unstable to sample (falls through sieve).

2. 50% of structural integrity lost within 5 – 30 seconds of immersion AND <10% remains after 5 dipping cycles.

3. 50% of structural integrity lost within 30 – 300 seconds (5 minutes) of immersion, OR <10% of soil remains on sieve after 5 dipping cycles

4. 10 – 25% of soil remains on sieve after 5 dipping cycles

5. 25 – 75% of soil remains on sieve after 5 dipping cycles

6. 75 – 100% of soil remains on sieve after 5 dipping cycles

**Figure 6.** Wet aggregate stability scores of soil aggregates at 0-15 cm depth, according to the NRCS slakes kit, for three cover crop trials in Central Valley in California, data for 2020. Error bars represent the standard error of the mean. Cover crop effect p-values obtained with ANOVA are presented for each site.

#### **Water Infiltration**



**Figure 7**. Soil water infiltration rate for two cover crop trials in the Central Valley of California, three years of cover crop use. Data collected in 2020. Different letters denote different groups according to Tukey test results for each site.

 The results indicate that the impact of cover crops on water infiltration is site-specific, with cover crops increasing water infiltration at the **Delta** site but not at **Mendota**. Unfortunately, due to changes in agricultural management practices at the **Sutter** site, infiltration measurements were not possible at the end of the study. At the **Mendota site** (**Figure 7A)**, cover crops did not (p= 0.53) change the infiltration rate compared to the control, with median infiltration rate values of 2 cm/min. In contrast, at the **Delta** site(**Figure 7B**), cover crops significantly improved water infiltration (p<0.001), with the CC plots having an average water infiltration rate of 3.47 cm/min and the fallow control having an average infiltration rate of 0.43 cm/min. There was a 703 % increase in water infiltration with the use of summer cover crops at this site, demonstrating the potential of cover cropping to enhance soil water management in certain environments. In addition, at the Delta site, the grower observed differences in subsequently planted small grains, with seedlings in the cover crop plots emerging about five days earlier than seedlings in the control plots. These observations suggest that cover crops enhanced the soil's ability to percolate

water, likely due to the presence of root systems that promote water infiltration. Similar effects have been reported in the same region of the San Joaquin Valley, where the combination of cover crops and no-tillage had the fastest infiltration rate compared to tilled and fallow plots (Mitchell et al., 2017). This trend seems to persist over time compared to previous data collected by Herrero et al. (2001) at the same site. These results highlight the effectiveness of cover cropping in enhancing soil infiltration, which can be critical for managing water resources, especially in Mediterranean regions where water availability is a concern(Ferreira et al., 2021). The combined effect of channels created by cover crop roots and lack of disturbance from no-tillage explains the increase in water infiltration rate reported by Mitchell et al. (2017). In addition, the presence and increase in worm populations due to available food sources for the soil macrofauna (Johnson-Maynard et al., 2007) from cover crop residue can create burrows for water to infiltrate through faster preferential pathways through the soil profile(Capowiez et al., 2009).

### **4. Conclusions**

Overall, this study underscores the importance of considering site-specific factors when evaluating the effectiveness of cover crops in improving soil health in the Central Valley in California. Soil carbon stocks showed mixed responses, with some sites experiencing reductions in carbon stocks, particularly in the upper soil layers, while others saw increases at greater depths. POXC and total nitrogen did not show significant changes across most sites, possibly due to the rapid decomposition of cover crop residues and interactions with other management practices like compost addition. Similarly, soil pH and EC remained largely unaffected, with EC being influenced by irrigation at the Delta site. Wet aggregate stability showed no consistent improvement across sites. However, water infiltration improved significantly with the use of a summer cover crop at the Delta site, highlighting the potential of cover crops to enhance soil water dynamics under certain conditions.

While cover crops offer potential benefits such as increased deep soil carbon sequestration and improved water infiltration, their impact on other parameters like soil nitrogen, POXC, pH, and aggregate stability may be limited or variable depending on the site conditions and management practices. These results suggest that cover crops should be integrated into broader soil management strategies, considering local environmental conditions and long-term goals for soil health and productivity. These findings emphasize the need for long-term studies to focus more on deeper soil depths to fully understand the cumulative effects of cover crops on soil health and to develop tailored management strategies that maximize their benefits while minimizing potential drawbacks.

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# **Appendix**

**Table A1** *- Soil organic carbon and nitrogen concentration (%), standard errors and bulk densities (g cm-3 ) are shown for all long and short-term sites. Mendota: cover crop (CC), no cover crop (NO), Sutter: no cover crop (Control), Low cover crop rate (Low CC), High cover crop rate (High CC), WSREC: : cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT), no cover crop standard till (NOST)*.

Site	Depth (cm)	Treatment	%C $(\pm$ SE)			%N $(\pm$ SE)			<b>Bulk</b> Density $(g cm-)$ $^{3}$
	$0 - 15$	<b>CCNT</b>	1.2	$\pm$	0.23	0.13	Ŧ	0.02	1.23
		<b>CCST</b>	1.14	$\pm$	0.09	0.13	$\pm$	0.01	1.09
		<b>NONT</b>	0.93	$\pm$	0.11	0.11	$\pm$	0.01	1.26
		<b>NOST</b>	0.9	$\pm$	0.07	0.11	$\pm$	0.01	1.31
	15-30	<b>CCNT</b>	1.11	$\pm$	0.18	0.12	$\pm$	0.02	1.6
		<b>CCST</b>	0.88	$\pm$	0.07	0.1	$\pm$	0.01	1.6
		<b>NONT</b>	0.98	$\pm$	0.12	0.11	$\pm$	0.01	1.6
		<b>NOST</b>	0.79	$\pm$	0.07	0.09	$\pm$	0.01	1.6
	30-60	<b>CCNT</b>	0.51	$\pm$	0.11	0.06	Ŧ	0.01	1.45
		<b>CCST</b>	0.43	$\pm$	0.03	0.05	$\pm$	0	1.45
		<b>NONT</b>	0.36	$\pm$	0.04	0.04	$\pm$	0	1.45
		<b>NOST</b>	0.38	$\pm$	0.02	0.05	$\pm$	0	1.45
		<b>CCNT</b>	0.42	$\pm$	$\mathbf 0$	0.04	$\pm$	0	1.45
	60-90	<b>CCST</b>	0.54	$\pm$	0.13	0.06	$\pm$	0.01	1.45
		<b>NONT</b>	0.45	$\pm$	0.04	0.04	$\pm$	0	1.45
		<b>NOST</b>	0.43	$\pm$	0.06	0.04	Ŧ	0	1.45
	$0 - 15$	CC	0.59	$\pm$	0.05	0.03	$\pm$	0	1.6
		<b>NO</b>	0.67	$\pm$	0.04	0.04	$\pm$	$\mathbf 0$	1.6
		CC	0.96	$\pm$	0.02	0.06	$\pm$	0.01	1.7



**Table A2** - Change in carbon stocks after 3 years of treatment at each site, by depth. Mendota: cover crop (CC), no cover crop (NO), Sutter: no cover crop (Control), Low cover crop rate (Low CC), High cover crop rate (High CC). Significant differences among treatments are highlighted in green.



after 3 years of treatment at each site, by depth. Mendota: cover crop (CC), no cover crop (NO), Sutter: no cover crop (Control), Low cover crop rate (Low CC), High cover crop rate (High CC). Significant differences among treatments are highlighted in green.

**Table A2** -

stocks

in

### **Chapter2 –**

# **Replacing Synthetic Nitrogen Fertilizer with Vermifiltration By-Products: Effects on Soil and Walnut Tree Health**

### **Abstract**

The global rise in food demand necessitates efficient and sustainable agricultural practices. While synthetic fertilizers have significantly boosted crop yields, their extensive use has led to environmental concerns and increased costs. Organic amendments, such as vermicompost, have demonstrated the potential to enhance soil health and decrease the dependency on synthetic fertilizers. Vermifiltration, an innovative vermicomposting technique, produces unique by-products that differ from traditional vermicompost and may impact soil health differently. This study investigates the effects of vermifiltration-derived vermicompost (VC) on soil health and tree growth in a commercial walnut orchard in Yolo County, California (USA), marking the first comprehensive examination of VC in orchard systems. Four treatments were applied: three rates of VC replacing 7%, 14%, and 20% of synthetic nitrogen fertilizer, and a control receiving 100% synthetic nitrogen. Over two years, VC application significantly increased soil organic carbon and total nitrogen at 0-15 cm depth and improved extractable phosphorus and zinc levels without altering soil pH and electrical conductivity. However, VC did not significantly impact soil microbial diversity. Walnut yield remained consistent across all treatments, indicating that VC can effectively replace synthetic nitrogen fertilizers while maintaining productivity. This study highlights the potential of vermifiltration-derived VC as a sustainable nutrient management strategy, promoting soil health and reducing synthetic fertilizer dependency in walnut orchards. Further long-term research is needed to fully understand the persistent effects of VC on soil health and microbial communities.

### **Introduction**

The global food demand is expected to increase by more than 50% by 2050, representing a significant global concern. Currently, hundreds of millions of people remain hungry, even though agriculture already utilizes almost half of the world's arable land (Searchinger et al., 2019). Therefore, there is a pressing need for efficient and sustainable methods to enhance agricultural productivity. Synthetic fertilizers have played a crucial role in increasing food supplies providing a fast and effective way to deliver nutrients to crops. However, there have been negative consequences to their extensive use such as the contamination of water supplies, an increase in nitrous oxide emissions, and nutrient imbalances in the soil, resulting in its degradation (Tripathi et al., 2020). Rising costs due to geopolitical conflicts and shifts in supply chains, combined with their environmental impact, have promoted the search for more sustainable alternatives (Lam et al., 2022).

Organic amendments, such as vermicompost, are often used to enhance soil health as part of sustainable agricultural practices. They improve soil structure, enhance water retention, and increase soil nutrient availability and organic matter (Aparna et al., 2014; Urra et al., 2019). Agricultural practices produce substantial organic waste, including crop residues, animal manure, and other by-products. Transforming these organic wastes into nutrient rich vermicompost could significantly reduce the reliance on synthetic fertilizers, improve soil health and fertility and promote sustainable agricultural practices by recycling waste materials and minimizing environmental impacts.

Vermicompost (VC) is a type of compost produced using earthworms and microorganisms (Aira et al., 2006). During vermicomposting, earthworms break down organic matter, stimulating biochemical decomposition by their gut microbiota (Gómez-Brandón et al., 2011). Then, earthworm casts suffer further biochemical degradation, resulting in a nutrient-rich organic material (Lim et al., 2015). The process yields vermicompost and earthworm biomass (Lim et al., 2015).

Vermicompost has gained popularity due to its unique characteristics and effects as a soil amendment, like higher nutrient content and distinct microbial community compared to other types of compost (Adhikary, 2012; Arancon et al., 2003). It is also produced faster than other compost due to the aid of epigeic species of earthworms that live on the surface and mix and break down big pieces of organic substrates (C. A. Edwards et al., 2010; Sim & Wu, 2010).

Vermicompost has been extensively studied and widely recognized for its beneficial impacts on soil health, enhancing soil structure, nutrient availability, and microbial activity (C. A. Edwards et al., 2010; Lazcano et al., 2011). Singh et al. (2008) found that vermicompost can replace synthetic fertilizers while reducing nutrient-derived and soil-borne diseases in strawberry cultivars. Arancon et al. (2003) vermicompost decreased plant parasitic nematodes and increased crop productivity in grape and strawberry crops. It has also been reported that VC contains growth factors that can benefit the crop and affect plant biomass allocations, improving fruit nutrient content in crops like tomatoes, grapes, potatoes, cucumbers and strawberries (Adrian Broz et al., 2017; Ali Reza Ladan Moghadam et al., 2012; Johann G. Zaller & Zaller, 2007; Rola M. Atiyeh et al., 2002; Ya-Nan Zuo et al., 2018). Additionally, studies have shown that applying VC can modify the microbial composition within the rhizosphere or growing mediums, reducing soil-borne plant diseases caused by fungi and bacteria (Simsek-Ersahin, 2011).

Vermicompost offers several advantages over traditional compost regarding soil health, plant growth, fruit quality, and yield. While both VC and compost have been found to improve soil structure, enhance nutrient availability, and promote microbial activity, VC is particularly rich in beneficial microbes and enzymes that arise from the earthworm's gut, which can accelerate nutrient cycling and improve plant nutrient uptake (Adhikary, 2012; Verma et al., 2024). Studies have shown that VC can significantly boost plant growth and yield due to its higher concentration of readily available nutrients and plant growth hormones (Syarifinnur et al., 2022; Uz et al., 2016). Additionally, VC has been found to improve soil water retention more effectively than regular compost, leading to increased crop drought resistance (Doan et al., 2015). Furthermore, VC's finer texture, due to worm activity that contributes to substrate aeration and breakdown, results in a higher nutrient content and lower pathogenic load that can provide more immediate benefits to plants, whereas traditional compost may take longer to break down and release nutrients (Islam et al., 2016; Lleó et al., 2013). Both amendments offer valuable benefits, but VC can provide more rapid and enhanced effects on soil health and plant productivity.

Although vermicomposting has traditionally utilized solid waste, the principles of vermicomposting can also be applied to treat liquid waste in a novel process known as vermifiltration. In this process, organic wastewater is applied to a bed of organic media (e.g., woodchips, sawdust, straw) inoculated with epigeic earthworms (e.g., *Eisenia fetida* and *Eisenia andrei*) (L. Zhao et al., 2010). The liquid waste forms a film around the media that is used by the earthworms and microorganisms, promoting biochemical decomposition. This process accelerates the stabilization of organic matter, modifies its physical and biochemical properties, and reduces the organic load of the wastewater (Arora et al., 2020; Lai et al., 2018). Vermifiltration has effectively reduced chemical oxygen demand, biological oxygen demand, and total suspended solids in diverse effluents (Permana et al., 2024). However, the by-products of vermifiltration remain largely unexplored and can have different physicochemical characteristics compared to traditional VC. These differences arise due to the distinct operational mechanisms of vermifiltration, including continuous water flow, and the presence of the bedding material. These differences can alter the microbial communities, carbon:nitrogen ratio, and nutrient composition of the resultant by-products (R. Singh et al., 2017). To our knowledge, only one study has investigated the effects of vermifiltration by-products on annual crops under semi-arid regions with a Mediterranean climate (Malal et al., 2024). This study illustrates that vermifiltration by-products can increase total soil carbon and nitrogen and enhance the activity of C-N-P cycling soil enzymes. This type of VC can be used as a sustainable management approach to recycle nutrients and enhance soil health.

As large-scale industrial vermifiltration becomes more common in intensive agricultural regions such as CA (Dore et al. 2022), large amounts of vermifiltrate vermicompost will become available to farmers. Therefore, there is an urgent need to better understand the impacts of this organic fertilizer on soil health and its feasibility to replace synthetic nutrient inputs. In particular, the effects of vermicompost on soil carbon, nutrient availability, soil microbial communities, and crop productivity need to be investigated.

Vermicompost and synthetic nitrogen (N) fertilizers differ significantly in their effects on soil health, nutrient cycling, and plant growth. Vermicompost is a rich source of organic carbon, organic nitrogen, and nutrients(Adhikary, 2012), which are absent in synthetic N fertilizers. The organic C in vermicompost promotes soil microbial activity and enhances soil organic carbon content, contributing to improved soil structure, soil aeration, increased waterholding capacity, and increased carbon cycling(Qasim et al., 2023). Additionally, vermicompost is rich in beneficial soil microbes like nitrogen fixing and phosphate solubilizing bacteria(Mishra et al., 2022), that can increase plant nutrient availability, supplying a broad spectrum of micronutrients, such as iron, zinc, and manganese, which are important for plant health and are typically not provided by synthetic N fertilizers(Singh et al., 2010; Yadav et al., 2016). This microbial activity supports plant growth and helps suppress soil-borne diseases, thereby reducing reliance on chemical pesticides (Arancon et al., 2003; Mishra et al., 2022). Furthermore, vermicomposts are generated through

sustainable waste management practices that transform organic waste into a valuable resource, thus contributing to a circular economy(Hajam et al., 2023). In contrast, synthetic N fertilizers primarily supply readily available nitrogen for plant uptake, which can easily be applied and managed but can lead to nutrient imbalances and soil acidification(Nemadodzi et al., 2017), potentially affecting soil carbon stability and increasing nitrogen leaching, which can have negative environmental impacts(Tripathi et al., 2020).

Walnuts are among the most consumed commercially grown tree nuts in the world and many health benefits have been claimed for their consumption, including reduced risk of cardiovascular disease, coronary heart disease, type II diabetes treatment, and prevention of certain cancers (Hayes et al., 2016) .With 689,461 million tons produced in 2023-2024, the USA is the world's second largest producer of walnuts after China (USDA, 2024). Within the USA, California is responsible for more than 90% of the production (National Agricultural Statistics Service,2024). The Data from USDA's National Agricultural Statistics Service (NASS) (*Walnuts Grown in California; Decreased Assessment Rate*, 2023), reported that the value of the walnut industry in California was approximately \$1.069 billion for the 2019–2020 through 2021–2022 marketing years. California has approximately 4,400 walnut growers and 155,800 hectares of walnut orchards. In this context, incentives exist for growers to use organic fertilizers and soil amendments, through the California Department of Food and Agriculture (CDFA) Healthy Soils Program(*CDFA - OEFI - Healthy Soils Program Incentive Grants*, 2024). This program provides financial assistance to farmers and ranchers to implement conservation management practices that improve soil health, sequester carbon, and reduce greenhouse gas emissions. The Healthy Soils Program aims to promote longterm adoption of these practices by providing grants and technical assistance. Therefore, there is a vital need to study and implement sustainable agricultural practices such as vermifiltration-derived VC that could promote sustainable walnut production with reduced fertilizer inputs in this region.

The objective of this study is to understand the impacts of vermifiltration-derived VC as a replacement for synthetic nitrogen fertilizer, specifically its impacts on soil and tree health. We hypothesize that VC produced through vermifiltration can successfully replace synthetic nitrogen fertilizer, provide a carbon source to feed the microbial community, and provide nutrients to walnut trees, maintaining productivity. Moreover, we further hypothesize that there is an optimum rate of VC application that will effectively replace corresponding amounts of synthetic nitrogen, optimizing soil nutrient availability and uptake. In addition, we hypothesize that VC can be used as a soil health management strategy to increase soil organic carbon and enhance microbial diversity, which is crucial for nutrient cycling and soil health.

# **2. Material and Methods**

# **2.1 Site description and Experimental setup**

We performed a field experiment at a commercial walnut orchard in Yolo County, California (USA). The walnut orchard (*Juglans regia L*., 'Hartley' variety) had been established for 14 years. The soil type at the site is classified as Rincon, Silty Clay loam (USDA-NRCS, 2023). The baseline physicochemical properties of the soil at 0-15 cm depth are  $1.55 \pm 0.08$  % C, 0.172 $\pm$ 0.005 %N, soil pH is 6.72  $\pm$  0.11, electrical conductivity (EC) is 581  $\pm$  43  $\mu$ S/cm, and soil bulk density is 1.41  $\pm$  0.02 g/cm<sup>3</sup>. The soil at 15-30 cm depth has 0.67  $\pm$  0.03 % C, 0.097  $\pm$  0.002 %N, soil pH is 6.85  $\pm$  0.05, EC is 542  $\pm$  25  $\mu$ S/cm, and soil bulk density is 1.60 $\pm$  0.02 g/cm<sup>3</sup>. The walnut orchard was not tilled except for the incorporation of the vermicompost in this study. In the last 5 years the pruning waste was removed and burned to reduce inoculum from wood disease pathogens. No compost has been added to this site since the establishment of the orchard. Elemental sulfur was added to the soil at 2.2 tons/ha in 2014 to offset high bicarbonate irrigation water. The residues from harvest, corresponding to hulls, leaves, and twigs, were deposited back in the soil surface after harvest as part of the grower's management practices.

The VC used in the experiment was produced in a vermifiltration system operated by Biofiltro Inc., Hilmar, CA. The vermifilter utilizes dairy wastewater as feedstock. The vermifilter consisted of a concrete rectangular enclosure ( $49 \times 11 \times 1.5$  m) with earthworms (*Eisenia fetida*) within the top 30 cm of the filtering medium (woodchips in 2020 and almond shells in 2021). Dairy wastewater (influent consisting of liquid manure and urine) was sprinkled for 2 min on top of the filter every 30 min. The surface of the vermifilter was tilled every month (Dore et al., 2022). After 18 months, vermicompost (including earthwork casts and remaining filter medium) was harvested from the vermifiltration system without further processing, and used as an organic amendment in our experiment to study its effects on soil and tree health and walnut tree performance.

The VC was analyzed three days before application to define vermicompost application rates. Each year, rates were determined based on the VC plant-available nitrogen (PAN), which includes nitrate, 20% of ammonium, and 30% of estimated organic N mineralization in the first year. content to achieve the target nitrogen replacement percentages. Plant available nitrogen was calculated as Vermicompost characteristics for each year, which can be found in **Table 1.**







The experiment followed a randomized complete block design (RCBD) with four treatments and three replicates per treatment (vermicompost application rate), separated into three blocks. The four experimental treatments included: three different rates of vermicompost (VC) replacing 7, 14, and 20% of synthetic nitrogen fertilizer, and a control treatment that received 100% synthetic nitrogen fertilizer with no VC (Table 2). The experimental plots comprised 22 x 42 meters orchard sections and included 14 trees organized in four rows. The vermicompost was applied on 3.3 m wide strips between the tree rows in each plot. Vermicompost, including woody substrates from the filter material, was spread using a manure spreader and was incorporated 10 cm deep immediately after application. Buffer zones were established between plots to avoid mixing effects. Vermicompost was applied in the November  $11<sup>th</sup>$  of 2020 and April 20<sup>th</sup> of 2022, with nitrogen rates and total vermicompost application detailed in **Table 2.**





Top of Form

# **2.2. - Soil Sampling**

Annual soil sampling was conducted in the fall, precisely on December 20, 2021, and December 8, 2022, 14 and 8 months, respectively, after vermicompost (VC) application. In 2021, sampling was conducted only at 0-15 cm depth. The following year, in 2022, soil samples were collected from two depths: 0-15 cm and 15-30 cm. For each depth and plot, 10 subsamples were randomly taken and composited. The collected soils were air-dried, sieved to <2mm, and cleared of visible plant residues. Additionally, a subsample of the fresh soil collected from the 0-15 cm depth in 2022, sieved to <2 mm and was frozen at -80°C for future microbial analysis.

# **2.3. Walnut Harvest**

Harvest was done annually during the fall on October 14, 2021, and October 7, 2022. A mechanical tree shaker was used to shake the walnut trees, causing the ripe nuts to fall to the ground. Then, a mechanical sweeper was used to gather the nuts into windrows (long rows) between the trees. This step ensured that the walnuts were aligned for easier collection. Transects of 2 m were manually collected from the windrows in each plot. All the collected walnuts were separated from the hulls and oven-dried at 65 °C until the dry weight remained constant. The yield was calculated as a ton per hectare based on the dry walnut weight collected from the 2 m transects taken from each plot and multiplied by the plot area.

# **2.4. Leaf sampling**

The second year after the VC application, leaves were collected from the trees on July 22nd, 2022. Terminal leaflets from fully expanded spur leaves were selected, located 6-8 feet above the ground from around the tree from the middle row (James Beutel et al., 1879). Four leaflets per tree from ten randomly selected trees inside the plot (40 leaflets total) were cut and taken to the lab, where they were washed with DI water and dried for macro and micronutrient analysis.

# **2.5 Analysis Methods**

# **2.5.1 Soil Analysis**

Air-dried soil sub-samples, sieved to 2mm, were analyzed for total carbon and nitrogen percentage by dry combustion using an elemental analyzer (Costech Analytical Technologies Inc. model EAS32). Permanganate oxidizable carbon (POXC) was analyzed based on Weil et al., (2003). Briefly, 2.5g of air-dried soil was shaken for 2 min with 20 mL of 0.02M KMnO<sub>4</sub>, a 0.50 mL aliquot of the sample was diluted 100 times with DI, and the concentration of KMnO4 was determined colorimetrically at 550nm (Shimadzu Scientific Instruments Inc. model UV-1280). Three replicates per sample were analyzed, and results were accepted when the coefficient of variation (CV) was  $\leq$  20%.

Particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) were analyzed according to Cambardella and Elliott (1992). Briefly, 10 g of air-dried soil was shaken for 15 h with 30mL of 5 g/L sodium hexametaphosphate. Then, samples were passed through a 53-μm sieve, and small rinses with DI water were made to separate the fractions. The organic matter smaller than 53-μm was identified as MAOC, and POC was identified as larger than 53-μm. Each fraction was oven-dried for 24h at 50°C and analyzed for total organic C by dry combustion.

Soil pH and EC were analyzed in 1:2 soil: DI water slurries, using a pH/EC probe (Mettler Toledo, Columbus, OH, United States). Soil-available phosphorus was extracted by adding 50.0mL of 0.5M NaHCO<sub>3</sub> (pH=8.5) to 2.50 g of air-dried soil. Samples were shaken for 30min in a reciprocal shaker and filtered. A 40 $\mu$ L aliquot of soil NaHCO<sub>3</sub> extract was mixed with 20μL MA reagent in each well of the 96-well microplate and shaken for 1min, then 140μL aliquot of deionized water was added. Absorbance was read at 700nm using a microplate reader, and soil Olsen-P concentrations were calculated based on the standard curve (Song et al., 2019).

Soil samples were sent to the UC DAVIS Analytical Lab to measure the potential availability of soil micronutrients; Zn, Mn, Cu, and Fe using the diethylenetriaminepentaacetic acid (DTPA) extraction method (Lindsay and Norvell, 1978).

Moist soil sub-samples, sieved to 8 mm, were used to analyze potentially mineralizable nitrogen (PMN). Briefly, 10 mL of water was added to 8g of soil; the samples were purged with N<sub>2</sub> gas and incubated for 7 days at 37°C. Then, each sample was extracted with 0.67M K<sub>2</sub>SO<sub>4</sub>, and NH<sub>4</sub><sup>+</sup>-N was measured colorimetrically in the soil extracts. PMN was calculated as the difference between  $NH_4^+$ -N in the incubated and non-incubated samples.

Frozen soil samples were sent in 50 mL conical vials with secondary containment to EZBIOME laboratories (EZBIOME Inc. Gaithersburg, MD 20878, USA) for soil DNA extraction, 16S rRNA, and ITS sequencing for analysis of bacterial and fungal communities, respectively. Taxonomic profiling of 16S rRNA and ITS sequencing data was performed using the EzBioCloud microbiome taxonomy profiling platform (www.ezbiocloud.net) as described by Yoon et al. (2017). Forward and reverse paired-end reads were uploaded to the platform, which filters out low-quality sequences based on read length (<80 bp or >2,000 bp) and average Q values less than 25. Denoising and extraction of non-redundant reads were conducted using DUDE-Seq software. The UCHIME algorithm checked and removed chimeric sequences against the EzBioCloud 16S chimera-free database. Taxonomic assignment was executed using the USEARCH program, clustering sequencing reads into OTUs at 97% sequence similarity with the UPARSE algorithm. Functional profiles of the microbiome were estimated using the PICRUST algorithm, annotating functional abundance profiles based on the KEGG (Kyoto Encyclopedia of Genes and Genomes) orthology and pathway database. Subsampling, generation of taxonomy plots/tables, rarefaction curves, and calculation of species richness, coverage, and alpha and beta diversity indices were

performed using the EzBioCloud App. Microbial richness was measured using ACE and Chao1 indices. Based on taxonomic abundance profiles, beta diversity was calculated using Bray-Curtis distances. The data was extracted from the EZBio Cloud App and analyzed as described below.

### **2.6 Statistical analysis**

The data collected in this experiment were analyzed with RStudio (R Development Core Team, 2024). In this randomized complete block design (RCBD), experimental units (plots) are included into blocks, with each block containing all treatment conditions (Control, Low VC, Med VC, and High VC) to control for the variability between blocks, which ensures that the vermicompost treatment effect is not confounded by block spatial variability effects. To account for the RCBD structure, the data were analyzed using a linear mixed-effects model, with the "lmer" function from the lme4 package. In this model, VC rate was included as a fixed effect to estimate its influence on the response variable, while block was included as a random effect to control for variability between blocks. Models for each variable were generated for each year and each soil depth separately. The normality of model residuals was assessed using the Shapiro-Wilk test. A Q-Q plot of the residuals was also generated to visually inspect the distribution of residuals. The homogeneity of variance across treatment groups (VC rate) was verified using Levene's test, where VC rate was used as the grouping factor. An analysis of variance (ANOVA) using a significance level of  $\alpha$  = 0.05 was used to assess the significance of the fixed effect in the models. Post-hoc pairwise comparisons were performed to further investigate significant effects found in the ANOVA, focusing on differences between VC rates. Comparisons were initially conducted using Tukey's Honest Significant Difference (HSD) test. The results of the Tukey tests were reported with adjusted p-values to account for multiple comparisons. If Tukey's HSD test failed to detect significant differences between treatments, Fisher's Least Significant Difference (LSD) test was subsequently applied. This test provides a less conservative approach, increasing sensitivity to detect differences between VC rate means. The results of both the Tukey and LSD tests were reported with the corresponding p-values. The confidence level for all tests was set at 95% ( $\alpha$  = 0.05).

#### **3. Results**



### **3.1 Effects of the vermicompost treatments on Soil Carbon Cycling Indicators**

**Figure 1**. Total soil carbon stocks (Mg C/ha) for each treatment at A) 0-15 cm and B) 15-30 cm for all years collected in a walnut orchard amended with different vermicompost rates in two consecutive years. P values < 0.05 indicate strong evidence against the null hypothesis  $(H_0 = \mu_i = \mu_j)$  as determined by the ANOVA test of the RCBD.

This study investigated the effects of different rates of VC on various soil carbon indicators over two consecutive years of VC additions. We found that replacement of N fertilizer by increasing rates of VC increased total soil carbon after the first year of application (p <0.001). As shown in **Figure 1A**, in 2021 the medium and high rates of VC significantly increased soil carbon by 60% and 68%, compared to the control, at a depth of 0-15 cm. However, after the second application, in 2022, no significant treatment effects were observed at 0-15 cm (p=0.074) and 15-30 cm (p=0.896), as seen in **Figure 1B**.

A similar trend was found soil active C (POXC), which was increased significantly with the Med and High rates of VC application in 2021 (p< 0.001, **Figure 2A**). Nonetheless, there were no significant effects after the second application of vermicompost in 2022 at 0-15 cm (p=0.482) and 15-30 cm (p=0.125).

No significant differences in particulate organic carbon (POC) between treatments were detected at either soil depth analyzed (**Figure 3A)**. However, we observed a trend of POC increasing with VC rates at 15-30 cm after two years of vermicompost applications. There were no significant differences between treatments at depths 0-15 cm and 15-30 cm for MAOC (p=0.198 and p=0.053, respectively).



**Figure 2.** Permanganate oxidizable carbon (POXC) concentration in soil samples collected in a walnut orchard amended with different vermicompost rates in two consecutive years. Data was collected at A) 0-15cm and B) 15-30 cm. P values < 0.05 indicate strong evidence against the null hypothesis ( $H_0 = \mu_i = \mu_j$ ) as determined by the ANOVA test of the RCBD. Different letters over the plots indicate significant differences between treatments within each depth and year according to the Tukey Post-Hoc test.



**Figure 3**. A) Particulate organic carbon (POC) and B) mineral-associated organic carbon (MAOC) at 0-15 cm and 15-30 cm collected in a walnut orchard amended with different vermicompost rates after two consecutive years, data from 2022. P values > 0.05 indicate no strong evidence against the null hypothesis  $(H_0 = \mu_i = \mu_j)$  as determined as determined by the ANOVA test of the RCBD.

### **3.2 Effects of The Vermicompost Treatments on nutrient cycling indicators**

Application of increasing rates of VC, increased soil total nitrogen (TN) after the first year of VC application at 0-15 cm (**Figure 4A**, p<0.001), where the highest rate of VC has the highest TN compared to the control in 2021. After the second year of VC application, we observed a significant treatment effect (p= 0.029) on TN with decreasing N content with increasing VC

application rates. However, the Post hoc Tukey test showed no significant difference among groups. The LSD Fisher's test revealed that the control and low rate had higher TN than the medium rate, which had 8% less TN than the control. The VC treatments had no significant impact (p=0.536) on TN at 15-30 cm, as shown in **Figure 4B**. The low rate of VC significantly increased the soil content of potential mineralizable nitrogen by 28% compared to the control (PMN, p<0.001) (**Figure 5**).



**Figure 4.** Total Soil Nitrogen for all years at A) 0-15 cm and B) 15-30 cm depth collected in a walnut orchard amended with different vermicompost rates in two consecutive years. P values < 0.05 indicate strong evidence against the null hypothesis  $(H_0 = \mu_i = \mu_j)$  as determined by the ANOVA test of the RCBD. Different letters over the plots indicate significant differences according to the Tukey Post-Hoc test or Fisher's LSD test.



**Figure 5.** Potential mineralizable nitrogen (PMN) at 0-15 cm after 2 years of VC application, data from 2022. P values < 0.05 indicate strong evidence against the null hypothesis  $(H_0 = \mu_i = \mu_j)$  as determined by the ANOVA test of the RCBD. Different letters over the plots indicate significant differences according to the Tukey Post-Hoc test.

The VC rates did not affect soil pH and EC at 0-15 cm. The results for pH are shown in **Figure 6A.** Overall, the pH was similar for all the treatments, including the control, and stayed at 6.5-7 during the study. We oserved no significant treatment effect on soil pH for 2021 (p=0.508) and 2022 (p=0.681). The results for EC are presented in **Figure 6B**; all the treatments, including the control, had an EC value between 500-600 µScm-1. We did not detect a significant effect between treatments for 2021 (p=0.689) and 2022 (p=0.509) at 0- 15 cm depth.

Extractable soil phosphorus significantly increased with the application of VC (p= 0.021). The highest rate of VC (High VC) demonstrated an 88% increase in available phosphorous compared to the control, as shown in **Figure 7**.



**Figure 6.** Soil EC and pH at 0-15 cm for two years of VC application collected in a walnut orchard amended with different vermicompost rates in two consecutive years. P values > 0.05 indicate no strong evidence against the null hypothesis ( $H_0 = \mu_i = \mu_j$ ) as determined by the ANOVA test of the RCBD.



**Figure 7.** Soil Available phosphorus concentrations measured in a walnut orchard amended with different vermicompost rates after two consecutive years, data from 2022 at 0-15 cm. P values < 0.05 indicate strong evidence against the null hypothesis  $(H_0 = \mu_i = \mu_j)$  as determined by the ANOVA test of the RCBD. Different letters over the plots indicate significant differences according to the Tukey Post-Hoc test.

We found that VC enhanced soil micronutrient concentration and decreased micronutrient variability among plots. Specifically for Zn, we measured a significant increase in soil Zn concentration (p=0.03), where the low and medium rates increased the Zn concentration by 0.2 mg kg<sup>-1</sup> and the high rate increased by 0.4 mg Kg<sup>-1</sup> compared to the control. As shown in **Figure 8**, the micronutrient concentration in soil amended with vermicompost had lower variability, as identified by smaller box sizes in the plot. In comparison, the control tends to have a higher variability among replicates.



**Figure 8.** Soil micronutrients concentrations collected in a walnut orchard amended with different vermicompost rates after two consecutive years of VC application at 0-15 cm, data from 2022. P values < 0.05 indicate strong evidence against the null hypothesis ( $H_0 = \mu_i =$  $\mu_j$ ) as determined by the ANOVA test of the RCBD. Different letters over the plots indicate significant differences according to the Tukey Post-Hoc test.



# **3.3 Effects of the Vermicompost Treatments on Soil Microbial Diversity**

**Figure 9.** Soil microbial alpha diversity indexes: A) ACE index results for bacteria, B) CHAO index for bacteria, C) ACE index for fungi, D) CHAO index for fungi measured in a walnut orchard amended with different vermicompost rates after two consecutive years of VC application, data collected in Fall 2022 at 0-15 cm soil depth. P values < 0.05 indicate strong

evidence against the null hypothesis ( $H_0 = \mu_i = \mu_j$ ) as determined by the ANOVA test of the RCBD. Different letters over the plots indicate significant differences according to the Tukey Post-Hoc test.

The alpha diversity of soil microbial communities was assessed using the ACE and CHAO1 indices, as illustrated in **Figure 9**. Adding VC at increasing rates did not significantly impact the overall richness of the soil microbial communities for bacteria and fungi after the twoyear period. Across the four treatments—control, Low A, Med A, and High A—there were no significant differences in both ACE (p=0.928) and CHAO1 (p=0.941) for bacterial richness indices. The same trend was observed for the fungal community, where no significant differences were obtained, specifically ACE (p=0.366) and CHAO1 (0.956).

Beta diversity was analyzed using the Bray-Curtis dissimilarity index to evaluate the differences in microbial community composition among the treatments. The results, presented in Figure 10, showed no significant differences in beta diversity for bacteria (p= 0.905) and fungi (p=0.956) among the control and the three VC rates. This suggests that applying VC at varying rates did not lead to distinct microbial community compositions after two years of VC additions.




**Figure 10.** Soil microbial beta diversity was measured with the Bray-Curtis dissimilarity index for A) bacteria and B) fungi measured in a walnut orchard amended with different vermicompost rates in two consecutive years, data for year 2022 at 0-15 cm soil depth. P values < 0.05 indicate strong evidence against the null hypothesis  $(H_0 = \mu_i = \mu_j)$  as determined by the ANOVA test of the RCBD. Different letters over the plots indicate significant differences according to the Tukey Post-Hoc test.

## **3.4 Effects of the Vermicompost Treatments on Tree Yield and Nutrition**

Our findings demonstrate the effectiveness of replacing synthetic N fertilizer by different VC rates in sustaining walnut yield. As depicted in **Figure 11**, in 2021 (year 1) the walnut yield remained consistent across all treatments (p= 0.762), including the control, averaging 10.2 tons ha<sup>-1</sup> . In 2022, the second year of the study, the walnut yield increased to an average of 13.5 tons ha<sup>-1</sup>, with no significant differences between VC rates and the control that received 100% synthetic nitrogen fertilizer (p=0.874).

In addition, all the trees for the different VC rates, including the control, had similar nitrogen content in their leaves. As described in **Figure 12**, there was no difference in leaf nitrogen content between the control and the VC rates (p= 0.22). The trees for all treatments were above 2.2% N after two consecutive years of VC application, the recommended nitrogen leave concentration for commercial walnut production in California(California Fertilization Guidelines for *Walnut*, UC Davis, 08-09-2024) .



Figure 11. Walnut yield in tons per hectare measured in a walnut orchard amended with different vermicompost rates in two consecutive years, data for Fall 2021 and Fall 2022. P values < 0.05 indicate strong evidence against the null hypothesis  $(H_0 = \mu_i = \mu_j)$  as determined by the ANOVA test of the RCBD. Different letters over the plots indicate significant differences according to the Tukey Post-Hoc test.



**Figure 12.** Walnut Leaf macronutrient concentration measured in a walnut orchard amended with different vermicompost rates after two consecutive years of VC application, data from 2022. P values < 0.05 indicate strong evidence against the null hypothesis  $(H_0 = \mu_i = \mu_j)$  as determined by the ANOVA test of the RCBD. Different letters over the plots indicate significant differences according to the Tukey Post-Hoc test.

Using vermicompost for two years influenced the leaf micronutrient concentrations in the walnut trees. Different rates of vermicompost significantly increased Mn levels in the leaves compared to the control (p=0.03). The low VC application rate increased leaf Mn by 27% compared to the control trees. There were no significant differences between the control trees and the trees amended with increasing VC rates for other micronutrients, but the VC rates exhibit less variability in the micronutrient content (**Figure 13**).



**Figure 13.** Walnut Leaf micronutrient concentration measured in a walnut orchard amended with different vermicompost rates after two consecutive years of vermicompost application, data from 2022. P values < 0.05 indicate strong evidence against the null hypothesis  $(H_0 = \mu_i = \mu_j)$  as determined by the ANOVA test of the RCBD. Different letters over the plots indicate significant differences according to the Tukey Post-Hoc test.

## **4. Discussion**

The results of this study provide valuable insights into the influence of different rates of vermicompost (VC) produced through vermifiltration on soil health, soil microbial community, and tree productivity and nutrition. Vermifilter-derived VC replaced different percentages of mineral fertilizer up to 20 % in a commercial walnut orchard. Our study tested the hypothesis that vermifiltration-derived VC could effectively replace synthetic nitrogen fertilizers in walnut orchards while maintaining tree productivity and enhancing soil health. The results supported part of our hypothesis, as VC application sustained walnut yields comparable to the control, demonstrating that VC can be a viable alternative to reduce synthetic nitrogen fertilizer use. However, contrary to our expectation, the highest rates of VC did not consistently enhance soil organic carbon or microbial diversity. Interestingly, while VC did increase soil total carbon and nitrogen after the first year, the second year of application revealed a decrease in nitrogen content with higher VC rates, suggesting a complex interaction between VC application and soil nutrient dynamics. These findings indicate that the optimal application rate for improving soil health indicators, such as organic carbon, soil macro and micronutrients, and microbial diversity, may require further investigation. Our study emphasizes VC as a sustainable fertilizer alternative, providing complete nutrients to the trees. It also emphasizes the need to carefully consider application rates to maximize soil health benefits.

## **4.1. Effects of the vermicompost treatments on soil health**

## **4.1.1 Soil Carbon Cycling Indicators**

The application of VC significantly enhanced total soil C levels in the first year, particularly at depths of 0-15 cm with medium and high VC rates, demonstrating 60% and 68% increases, respectively, compared to the control for total soil C. Vermicompost produced through vermifiltration contains decomposed organic material processed by earthworms and is rich in stable or more recalcitrant carbon compounds (García-Sánchez et al., 2017). As an organic carbon source, VC can contribute to forming stable soil aggregates that improve soil structure and protect carbon in the soil; it also serves as a nutrient reservoir to enhance crop productivity and act as a food source for soil biota. The increments in total soil C observed in our study confirm that VC is a promising strategy for increasing total soil C. However, the absence of significant differences between VC rates in SOC in the second year suggests that the impact on soil C is affected by other environmental factors. These findings are consistent with previous studies, which noted short-term increases in SOC following annual VC applications, with effects on SOC diminishing over time (Ghosh et al., 2021; Sarma et al., 2018).

The increase in soil C the first year after vermicompost application can be due to the incorporation of vermicompost in the fall, which could enhance ground cover growth and promote soil carbon accumulation. This occurs as the increased biomass from ground cover plants, including roots and above-ground residues, is incorporated into the soil, thereby enhancing the soil organic carbon pool(Moukanni et al., 2022). In the second year, we observed no difference among treatments, including the control. The second vermicompost application occurred during the spring, and the subsequent soil sampling happened eight months after the application. The application of vermicompost during the spring was intended to provide plant available nitrogen closer to the time of highest crop uptake when the walnut trees start developing leaves and buds. During this time, increased temperatures combined with irrigation may lead to faster mineralization of the vermicompost, causing higher soil respiration and decreasing differentiation among treatments.

In addition, It is important to note that in the case of our study, walnut orchard operations introduced biomass into the soil through tree leaves and walnut hulls deposited in the field after harvest, which could lead to less statistical differentiation between treatments for the second year. The soil organic matter content for our study site was 5.01±0.18 %, which is relatively higher than the reported values for other soils in the region, which, according to the Web Soil Survey, range from 0.75 – 2.40% in that area (USDA-NRCS, 2023). Although no differences were observed between treatments in 2022, the control and Low VC rates increased by 50% and 63% in soil total C, respectively, compared to the percentage reported for 2021. This reflects the combined impact of VC inputs (including woodchips, a resistant C substrate) and the previously mentioned walnut harvest biomass inputs, that can accumulate and explain the soil C increases in the control. A similar effect has been observed for other recalcitrant carbon sources like biochar, where there is an initial increase in SOC,

but then the carbon mineralization rate decreases for subsequent years, stabilizing the SOC content(Sarma et al., 2018; Yousaf et al., 2017).

Soil carbon exists in distinct fractions with different functions and characteristics. These organic carbon fractions contribute to nutrient cycling, soil structure, and long-term productivity. POXC is commonly interpreted to represent a labile fraction of SOC, easily oxidized by potassium permanganate, representing the active carbon pool that contributes to nutrient cycling (Weil et al., 2003). This fraction is a sensitive indicator of changes in soil organic matter in the short term (Margenot et al., 2017). We observed that VC increased POXC in the first year of application for the medium and high application rates. This increase is crucial for short-term soil fertility as it provides readily available nutrients for microbial activity and plant growth (Culman et al., 2012). These findings are consistent with both Sarma et al. (2018) and Ghosh et al. (2021), where vermicompost application resulted in a significant increase in labile carbon fractions, such as POXC, in the first year but did not show further increases in the second year. This pattern is attributed to the rapid mineralization and stabilization of the easily degradable organic matter introduced by vermicompost in the first year (Sarma et al., 2018). Ghosh et al. (2018) observed a similar trend where continuous vermicompost application over three growing seasons led to an initial boost in active carbon pools, followed by a plateau, indicating that the labile soil carbon reached an equilibrium that limited further increases. A similar observation has been reported for traditional compost in a semi-arid climate, where the different rates of compost from 4.5-13.5 tons/ha did not increase SOC after two years of inputs due to the slow carbon turnover but did increase POXC proportionally to the composting rate (Lazcano et al., 2023; Wong et al., 2023). In contrast, under arid climates, other studies have also found a 41.0–46.7% increase in SOC when compost is combined with synthetic NPK fertilizer application, compared to the full NPK fertilizer(Al-Suhaibani et al., 2020).

On the other hand, particulate organic carbon (POC) consists of partially decomposed plant and animal residues that are larger and less processed by microbes. POC plays a significant role in soil structure, improving soil aeration, water infiltration, and root penetration. It is also a slow-release nutrient source, enhancing long-term soil fertility (Cambardella & Elliott, 1992). By analyzing this fraction, we targeted the combined contribution of biomass residues introduced after harvest and the woodchips used as a substrate material in the vermicompost. This fraction was analyzed at the end of the study to evaluate the contribution of 2 years of VC applications. While the effects of VC were negligible at 0-15 cm, we observed a trend at 15-30 cm, where POC increased with increasing rates of VC. Our findings highlight the potential of vermicompost to improve POC. Similar findings were observed with the vermifilter-derived vermicompost application used over 2 years in annual cropping systems, where the percentage of particulate organic carbon increased for the two highest rates of vermicompost (Malal et al., 2024). In addition, after two years of vermicompost application at different rates, POC was increased by 5-21% compared to unamended control in a field study comparing the effects of vermicompost and biochar (Sarma et al., 2018). Vermicompost contains easily degradable organic matter, including partially degraded polysaccharides, that increase the labile fractions of soil organic carbon,

like POXC and POC ( Edwards et al., 2010; García-Sánchez et al., 2017; Pathma & Sakthivel, 2012; Atiyeh et al., 2002).

MAOC is considered the most stable SOC fraction tightly bound to soil minerals; it consists of small carbon molecules in organo-mineral associations protected from microbial degradation (Xu & Tsang, 2024). This fraction is important for long-term carbon sequestration due to its long turnover time, contributing to soil resilience degradation (Angst et al., 2023). In our study, we analyzed MAOC after two years of VC application, and we did not observe a pronounced effect at any depth in MAOC with increasing VC rates. Similar trends were observed in short-term studies with VC derived from vermifiltration under the Mediterranean climate for annual crop rotations (Malal et al., 2024), indicating that the applied C is not stabilized through mineral interactions in the short term. However, the observed POC trends following the application of VC suggest that there could be a longterm increase in MAOC since POCV can be microbially degraded in the soil and transformed into MAOC (Angst et al., 2023; Cotrufo et al., 2013; Kravchenko et al., 2019). According to Samson et al., (2020), adding a more processed or degraded carbon source with a high affinity for mineral surfaces can increase MAOC. Vermicompost, which has already been degraded by the worm's gut microbiota and contains more degraded organic matter (García-Sánchez et al., 2017), can potentially increase MAOC in the long term. Changes in MAOC due to the adoption of organic amendments and plant residue incorporation have been observed over the long term (Dămătîrcă et al., 2023). Due to its chemical characteristics and the results observed in our study, vermifilter-derived vermicompost has the potential to help stabilize soil carbon in the long term, providing a strategy for mitigating climate change. However, it is important to conduct additional studies over several years to evaluate the effects on different carbon fractions resulting from the long-term use of VC in soil management.

# **4.2. Soil Microbial Diversity**

Soil microbial diversity has been associated with higher agricultural productivity and resilience to environmental stress like drought (Giller et al., 1997; Gupta et al., 2022). Soil microbial communities perform different functions, such as nutrient cycling, disease suppression, and soil aggregate formation, all of which are important for soil health and resilience to climate change (Kallenbach et al., 2016; Verma et al., 2024). VC has been extensively studied because of its differentiated microbial community composition and function compared to other types of compost that result from the worm's gut activity and mesophilic degradation of organic matter during vermicomposting (Aira et al., 2015; Devi & Prakash, 2016; Domínguez et al., 2021; Khursheed Ahmad Wani et al., 2017; Pathma & Sakthivel, 2012; Pereira et al., 2023). In addition, vermicompost is a source of organic carbon and nutrients that can modify soil microbial functions (Domínguez et al., 2019; Lazcano et al., 2013). Therefore, we evaluated how vermicompost impacted soil microbial diversity after two years of vermicompost inputs.

By enriching the soil with organic matter and nutrients, vermicompost can create a more conducive environment for microbial communities to flourish, potentially resulting in increased microbial diversity and activity. An increase in microbial diversity can facilitate the multifunctionality of the microbial community in arid environments ( Zhao et al., 2023). The analysis of soil microbial alpha diversity using ACE and CHAO1 indices revealed no significant differences in microbial richness between the control and VC treatments for bacteria and fungi. Similarly, beta diversity analysis using the Bray-Curtis dissimilarity index showed no significant differences in microbial community composition among treatments. Our results suggest that while VC provides organic matter and nutrients to the soil, after two years of application, novel microbial populations may not be capable of establishing and displacing the native community of the bulk soil. Similarly, as previous results on soil health studies suggest, the native microbial community may be productive enough to resist inoculations by novel populations (Jones et al., 2021). These results are in contrast to the short term changes in community composition observed in applications of vermicompost in corn (Lazcano et al, 2013) and tomato (Zhao et al, 2019, Munoz-Ucros et al, 2020) plantations, which sampled community structure months after vermicompost application. Another study analyzed phospholipid fatty acids in a column experiment using various organic amendments like compost and discovered that they can alter microbial diversity within four months (Farrell et al., 2009). Our results suggest that these changes in community composition may be temporary and the dominant community may return following a longer period of time after the application of vermicompost or may be controlled by other carbon inputs like ground cover residues and residues from walnut harvest. Still, most of the available information is reported for lab incubations or mesocosm under controlled conditions and short periods of time(Pérez-Piqueres et al., 2006). The sampling time of our study relative to vermicompost application and sampling the bulk soil instead of the rhizosphere may have influenced the results observed; sampling during several seasons can better detect changes in the microbial community structure (Smit et al., 2001).

A field study under semi-arid conditions comparing manure compost to an unamended control found that the season impacted the growth and dynamics of the microbial community (Watts et al., 2010). Additionally, using date palm waste compost has been found to impact soil microbial diversity significantly and promote fungal growth when measured during the barley growing season(Ghouili et al., 2023). While, Cherif et al.(2009) found that after five years of using a combination of compost and chemical fertilizer, the microbial diversity of the soil was not affected in an arid region. These studies suggest that compost and vermicompost can enhance microbial communities in arid and semi-arid regions in the short term and that shifts in the microbial community may be seasonal or happen closer to the input of the organic amendment.

Field studies are needed to analyze the microbial diversity at different times after vermicompost application. In addition, we recommend the study of key microbial functions through metagenomic analysis (Kim et al., 2022) or microbial activity for a more in-depth understanding of the effects of vermicompost on microbial community structure and functioning.

# **4.3 Soil nutrient cycling indicators**

Soil total nitrogen (TN) is an important indicator in determining soil health, as it reflects the soil's capacity to provide this nutrient essential for plant growth and predicts yield (Gale et al., 2006). Our study replaced different percentages of mineral fertilizer with different rates of VC to evaluate the potential of VC to supply nitrogen to the walnut trees. Vermicompost application increased TN in the first year at 0-15 cm depth, with the highest rate showing the most substantial effect. This increase in TN persisted into the second year, although differences among treatments were not evident according to the Post-hoc test. This persistence highlights the potential of vermicompost to enhance nitrogen availability by supplying an organic nitrogen source that can be mineralized over time. These findings align with other studies indicating that vermicompost can enhance soil nitrogen content, improving soil fertility (Ali Reza Ladan Moghadam et al., 2012; Arancon et al., 2003; Malal et al., 2024). Furthermore, potential mineralizable nitrogen (PMN) increased significantly with the lowest rate of vermicompost, suggesting that even minimal applications can improve nitrogen availability. The increase in PMN only for the low rate of vermicompost could be explained by the low initial availability of mineral N, causing the soil microbes to mineralize N from the organic N pool. At the same time, the medium and high application rates had a higher initial inorganic N content, which as it has been previously evidenced in other studies does not promote N mineralization (Lazicki et al., 2020). Another potential mechanism could be that the high amount of labile C (POXC) in the high and medium application rates slowed down N mineralization, causing a reduction in PMN, as observed in other studies for treatments with high labile C content (Mallory & Griffin, 2007; Tyson & Cabrera, 1993).

Phosphorus is a macronutrient that plays a significant role in the growth and development of walnut trees. Vermicompost significantly increased extractable soil phosphorus, particularly at the highest application rate, which resulted in an 88% increase compared to the control. This finding suggests that VC application can significantly enhance the availability of this phosphorus. It has been estimated that one ton of harvested walnuts removes approximately 1.95 kg of phosphorus from the field (Demirbaş, 2002; Lavedrine et al., 2000); therefore, replenishment of soil phosphorus and appropriate pH management will be necessary to promote phosphorus availability for the lifespan of the walnut orchard, with trees increasing the phosphorus need with maturity (Simon et al., 2023). Further research is required to determine whether using VC at a rate higher than 7 tons per hectare is financially feasible as a phosphorus management strategy. It is also uncertain whether the incorporation of lower rates of VC will have a cumulative effect on soil phosphorus levels over time, justifying the use of VC as a long-term phosphorus management method. There is evidence that vermicompost performs better as a phosphorus fertilizer strategy than standard compost. Oo et al. (2013) found that when comparing vermicompost against traditional compost, extractable phosphorus was higher in vermicompost treatments in saline and non-saline soils in a casava plantation, with both organic amendments having higher extractable phosphorus than the NPK control. Similar findings were obtained by Asrin et al. (2019), where vermicompost had increased the value of the available P content of the soil by 15.45 mg/kg, greater than the application compost by 13.82 ppm compared to an unamended control. The presence of phosphorus-solubilizing microorganisms in worm casts, along with the action of earthworm gut phosphatases, further enhances phosphorus availability in vermicompost (Nsiah-Gyambibi et al., 2021). However, since vermicompost application is planned to meet the walnut trees' nitrogen demand, this could result in an over-application of phosphorus. It is advised to carefully select rates to avoid excessive phosphorus inputs, which could lead to phosphate leaching and cause environmental problems (Lazcano et al., 2023).

In our study, vermicompost enhanced soil micronutrient levels, specifically zinc (Zn), and reduced variability in micronutrient content among plots. Soil Zn levels increased with increasing VC rate. Zinc is the most common nutrient deficiency in young orchards in California, decreasing tree growth and yield (Gordon et al., 2024). Our results demonstrate that VC can be used as an effective nutrient management strategy due to its high organic matter content, which can supply and chelate Zinc and make it available for tree uptake. In addition, we observed minor nutrient variability between trees in all the VC treatments compared to the control. This effect was not rate-dependent, indicating that even lower rates of VC can stabilize micronutrient availability. These observations are consistent with the literature showing VC's ability to increase soil nutrient content by being a nutrient source without significantly affecting soil pH (Lim et al., 2015; Malal et al., 2024). Similarly, standard compost and compost tea have been found to enhance the availability of nutrients like nitrogen, phosphorus, and potassium in the soil in semi-desert climate after two years of application (Hakimi et al., 2024.).

High soil salinity can reduce crop productivity (Ruiz-Lau et al., 2020) and could be a concern when applying fertilizers. Our study found that vermicompost derived from vermifiltration did not significantly change soil salinity, as indicated by the soil electrical conductivity. This finding aligns with similar observations from a related study in the same region, which reported no significant changes in soil EC when vermifilter-derived vermicompost was used in an annual crop system with subsurface irrigation (Malal et al., 2024). This consistency across studies suggests that dairy waste vermifiltrate is not particularly high in salts, making it a viable option for soil amendment without the risk of increasing salinity. In contrast, studies involving different types of VC obtained varied results, with some researchers reporting a decrease in soil salinity and recommending VC as an alternative method to improve saline or sodic soils (Hafez et al., 2020; Oo et al., 2015; Xu et al., 2016) and, other authors reporting EC increases with vermicompost application rate due to the high soluble salt content (Akhzari et al., 2015; Gopinath et al., 2008). These discrepancies highlight the influence of the original feedstock and processing methods on the salt content of vermicompost. The absence of a significant increase in soil EC in our study suggests that vermicompost derived from dairy waste vermifiltration could be a safer alternative for arid and semi-arid regions concerned about soil salinity.

Soil pH at this site was managed by adding sulfur, a standard practice to prevent phosphorus deficiencies and ensure proper nutrient availability for the walnut trees. The application of vermicompost did not alter the soil pH, regardless of the rate applied. Other studies that have investigated the effects of vermicompost on soil pH have found that it tends to increase soil pH in acidic soils (Al-Maamori et al., 2023; Liu et al., 2019) and decrease pH in alkaline soils (Uz et al., 2016). No other studies have been conducted with vermicompost combined

with a pH management strategy, like the addition of sulfur. Our results demonstrated that vermicompost's influence on pH is reduced when other pH management strategies are in place.

# **4.4 Tree Yield and nutrition**

The application of VC maintained walnut yield at levels comparable to the control, which received 100% synthetic nitrogen fertilizer. Yields averaged 10.2 tons/ha in the first year and increased to 13.5 tons/ha in the second year. The yield consistency across treatments indicates that vermicompost can be a viable alternative to reduce synthetic fertilizer use, providing sufficient nutrients to sustain crop yield. We observed that VC rates supplied adequate nitrogen to the trees, with no significant differences in leaf nitrogen content between the control and vermicompost treatments. The nitrogen content of the tree leaves was above 2.5%, which is within the recommended levels of 2.2-3.2% N for walnut trees (Simorte et al., 2001).

Similar to traditional compost, vermicompost is a comprehensive source of nutrients that can be mineralized slowly over time, supporting crop growth (Adhikary, 2012; Rajbir Singh et al., 2008; Ya-Nan Zuo et al., 2018). Typically, the combined use of vermicompost or compost with synthetic fertilizer has been reported to increase yield due to the slow release of nutrients and decreased nutrient loss during the high crop uptake period (Al-Suhaibani et al., 2020). For example, Manivannan et al.(2009) found a 1.6-fold increase in bean (*Phaseolus vulgaris*) production when using vermicompost combined with synthetic fertilizer compared to only synthetic fertilizer. In addition, other studies have reported an increase in yield with the short-term use of combined synthetic fertilizer and compost for maize and faba bean (Al-Suhaibani et al., 2020), 60% yield increase for Barley (Agegnehu et al., 2016), and, strawberry ((Adrian Broz et al., 2017; Arancon et al., 2003; Ya-Nan Zuo et al., 2018). Contrary to these observations, in our study, combining synthetic fertilizer and vermicompost did not increase yield compared to the control (100% synthetic fertilizer). Our observations align with a meta-analysis that studied the yield response of different crops to soil organic amendments and concluded that the yield benefit of organic amendments was lower in arid regions and for fruiting crops, like walnuts (Wortman et al., 2017).

It has been reported that compost with C: N ratio higher than 30 can lead to a decrease in yield due to nitrogen immobilization (Choi et al., 2001; Giannakis et al., 2014; Lazicki et al., 2020). However, in our study, the vermifilter-derived vermicompost did not result yield or nutrient reductions despite its C: N ratio of 39. This can be attributed to the unique properties of the vermifilter-derived vermicompost, which has a high C:N ratio due to the presence of woodchips used as a substrate during vermifiltration but is very rich in macro and micro nutrients (Permana et al., 2024). The woodchips are covered in a worm-cast biofilm, and worm casts have been reported to have a high surface area (Domínguez et al., 2019, 2021; Lai et al., 2018), making them more susceptible to microbial colonization. The vermicompost has a high nitrate content, making it an ideal amendment to replace synthetic nitrogen fertilizer in spring when walnut trees have a high nitrogen demand. Nevertheless,

the effect of the residual woodchips on nutrient immobilization for subsequent years after vermicompost application still needs to be assessed.

Additionally, vermicompost application influenced micronutrient variability in tree leaves, significantly increasing manganese (Mn) levels compared to the control. The vermicompost used in this study is derived from dairy waste, which has been reported to contain a diverse array of micronutrients, including Mn(Aremanda et al., 2023). Manganese is an essential micronutrient because it is necessary for photosynthesis (Messant et al., 2023); it is also a cofactor for various enzymes involved in plant metabolism, including those responsible for nitrogen assimilation and synthesis of fatty acids (Schmidt & Husted, 2019). Previous research supports these findings, indicating that vermicompost can enhance crop yield and nutrient content and serve as an effective organic amendment (Arancon et al., 2004).

Interestingly, the variation in leaf micronutrient content between the trees sampled decreased for vermicompost treatments compared to the control trees, which could lead to easier nutrient management in the long term. Vermicompost has been shown to enhance macro and micronutrient levels in various crops across different climates when used for longer periods of time. For example, Tejada & Benítez (2020) conducted a five-year study that revealed the concentration of N, P, K, Ca, and Mg increased in the leaves of olive trees grown in a Mediterranean climate. Short-term effects on nutrient concentration have been observed in annual crops from different sources of vermicompost. (Al-Maamori et al., 2023; Kumar et al., 2018; Sahariah et al., 2020; Simorte et al., 2001). However, it takes longer for tree crops to display significant effects, which are more noticeable in younger trees or seedlings (Ozdemir et al., 2019).

The uptake of plant macro and micronutrients from vermicompost is generally higher compared to traditional compost (Balemi, 2017; Jakubus & Michalak-Oparowska, 2022; Sharma et al., 2011), which can be attributed to several factors related to the vermicomposting process and the mechanisms involved in nutrient availability. The nutrient release during vermicomposting is more efficient, as nutrients are converted into soluble and readily available forms for plant uptake due to the activity of earthworms (Lim et al., 2015; Mistry et al., 2015). In addition, vermicompost provides high particulate surface areas that provide many microsites for microbial activities and retention of nutrients ( Singh et al., 2008). In a greenhouse pot experiment comparing the use of compost and vermicompost at different rates with and without synthetic nitrogen fertilizer for corn growth, it was found that the optimal concentrations of plant Fe, Zn, Cu, Mn, N, P, K, Ca, and Mg were achieved with 3% vermicompost addition, as opposed to higher rates of compost. In addition, the same study found a decrease in yield due to high Zn levels in the compost treatments (Kalantari et al., 2011). These observations can be explained by the plant growth-promoting hormones and enzymes in vermicompost, which facilitate nutrient absorption (Kumar et al., 2018; Rajbir Singh et al., 2008). Our study presents groundbreaking results by successfully substituting synthetic nitrogen fertilizer with vermifilter-derived vermicompost and providing a comprehensive source of nutrients to the trees.

## **5. Conclusion**

The application of vermicompost produced through vermifiltration to a walnut orchard showed benefits for soil health, enhancing soil organic carbon, nitrogen availability, and soil phosphorus and Zinc levels. Contrary to our hypothesis, the effects on soil health, yield, and tree nutrition were variable depending on the rate of vermicompost. Soil organic carbon, total nitrogen, and available phosphorus and Zinc are enhanced with vermicompost rates higher than 4 tons/ha. While the 2 tons/ha rate increased potential mineralizable nitrogen and leaf Mn content. In addition, the effects of vermicompost on soil health parameters are variable over time due to interactions with other management practices like biomass inputs from the walnut harvest operations and the time of vermicompost application.

After two years, the microbial community in the bulk soil appears not to be affected by the inputs of vermicompost, maintaining its diversity and composition. This could be due to the extended sampling time after applying vermicompost and its interaction with other management practices. Vermicompost can increase tree nutrition and sustain yield, offering a viable alternative to reducing synthetic fertilizer use.

Therefore, our research indicates that vermifilter-derived vermicompost can serve as a valuable organic fertilizer in tree orchards, offering a sustainable alternative to replace up to 20% of mineral nitrogen fertilizer. By incorporating vermicompost into fertilization practices, growers can potentially improve soil health, enhance tree growth, increase yields, and promote sustainability in orchard management in Yolo County, which could be extrapolated to similar environmental conditions in California. While our study provides the proof of concept for utilizing vermicompost as a replacement for synthetic nitrogen fertilizer, long-term studies are needed to fully understand the full effects of vermifilter-derived vermicompost and the impact of the residual woodchips from the vermifiltration process, that increase the C: N of the amendment, and would likely impact soil health and microbial communities.

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# **Evaluating the Predictive Capability of Saturated Paste for Soil Bulk Density in Annual Cropping Systems in California**

#### **ABSTRACT**

Soil bulk density (BD) is important for measuring changes in soil chemical and physical and biological properties; however, the measurement is tedious to collect and requires specialized equipment. Database measurements for soil surface BD do not always correspond to present field conditions as field management can alter BD in time. Saturation percentage (SP) is a routine lab measurement. The objectives of this study are to understand if: 1) a relationship between BD and SP can be developed; 2) to build a model that predicts BD based on a routine low-cost lab analyses. We collected 83 soil samples from different experimental sites around California's Central Valley. At each site, BD, soil organic matter (OM), and soil total organic carbon (SOC) were measured. A set of models were generated and were compared based on their AIC and adjusted R<sup>2</sup>. The best two models are presented in this paper and their accuracy and precision in estimating BD was further compared by calculating the root mean square error (RMSE) and the  $R^2$  of the predicted versus real values. We determined that a strong relationship between BD and saturation percentage exists ( $R^2$ = 0.70) and that a cubic model that includes SP and OM resulted in the best model to predict BD in California soils. Inclusion of additional data may further strengthen this model or make it applicable for other grower regions.

#### **1. INTRODUCTION**

Bulk density (BD) is the weight of soil relative to its volume. Bulk density is critical for evaluating changes in soil organic matter and chemical factors that are measured by weight in soil testing labs (for example soil carbon). However, because this measurement requires removal of an intact soil core from the field, accurate collection of BD in the field can be tedious, requires specialized equipment, and measurements can vary by the individual collecting the data. In the United States, BD of a field is included in the United States

101

Department of Agriculture Web Soil Survey (USDA WSS), which is a free, publicly available resource (Soil Survey Staff, 2023). However, BD can be modified by standard agricultural field operations (da Silva et al., 1997). Thus, USDA WSS values may not accurately reflect current soil BD measurements.

Saturation percentage (SP) is a routine lab measurement that quantifies the soil water content of a saturated soil and is used for evaluation of salinity, soluble salts, electrical conductivity, among other things (Gavlak et al., 2005). Most soil testing labs conduct SP measurements and analyses are not expensive. Previous work has correlated SP to soil texture (Stiven & Khan, 1966), and has found that soil total organic matter (OM) effects the reliability of the model (Mbagwu & Okafor, 1995). In addition, edaphic factors like field capacity and wilting point, which rely on both texture and soil structure, have been estimated from SP (Grewal et al., 1990). A pedotransfer function has been developed to predict plant available water holding capacity based on soil texture and soil organic carbon (Bagnall et al., 2021), indicating the opportunity to predict soil structural characteristics like BD from a combination of physical and chemical soil measurements.

Previous work has correlated or attempted to predict soil BD to other edaphic factors like soil texture, soil carbon, and OM (Chaudhari et al., 2013; Sakin et al., 2011). However, a connection has not been drawn between BD and standard analytical lab measurements like SP. As the method for quantifying SP requires saturating all soil pore space with water, we hypothesized that it could be used to improve the model for predicting BD. However, because it does not require the removal of an intact soil core, it was unclear how accurate the relationship would be. Thus, models that included other edaphic measurements, such as OM and total carbon were generated. This project aims to evaluate SP as a predictor of actual BD in annual cropping systems in California's Central Valley. The objectives of this study are to understand if: 1) a relationship between bulk density and saturation percentage exists; 2) to build a model that predicts soil bulk density based on a routine low-cost lab analyses.

Different models were tested, and the best models were selected based on the adjusted  $R^2$ and AIC. The higher the adjusted  $R^2$ , the better the fit between the generated model and

102

the raw data. The AIC is an index of prediction likelihood and, thereby, the relative quality of statistical models for a given data set.

#### **2. MATERIALS AND METHODS**

#### **2.1 Soil Samples**

Four to ten soil cores were collected and mixed into a composite soil sample at 0-15 cm and 15-30 cm depths, or 0-30 cm depth, were collected from eight different agricultural sites in California's Central Valley, including the San Joaquin Valley and Sacramento Valley. Site characteristics are summarized in **Table 1.** Geographical site distribution can be observed in Figure 1. Bulk density was measured in each site by collecting 60 cm<sup>3</sup> intact soil cores from the middle of each sampling depth. Samples were dried and weighed. We collected a total of 83 soil samples from different plots under different treatments in experimental agricultural fields. Samples were collected from parts of the field with differing management practices compared to an unamended control.



**Figure 1.**Geographical distribution of sites across California.

## **2.2 Lab Analyses**

Saturation percentage was measured in all samples according to (Gavlak et al. 2005). Briefly, 200 ±0.05 g of air-dried soil, previously sieved to pass a 2-mm mesh, was placed in a weighted Erlenmeyer flask. Deionized water was added until a saturated paste was

obtained. Saturation point was reached when the paste had no remaining free-standing water, was glistening, slid off easily from a spatula and flowed when the flask is tilted at a 45° angle. Samples were left to equilibrated for 4 h and then more water or soil was added accordingly, until reaching saturation conditions. In addition, soil moisture (Pw) of the airdry samples was calculated by mass difference after drying a sub-sample of the air-dried soil at 105° C for 24 h.

Saturation percentage was calculated as follows:

$$
SP(\%) = \frac{Amount of water added (g) x 100}{mass of air dry soil (g) / (1 + Pw)}
$$

$$
P_w = \frac{mass wet soil (g) - mass oven dry soil (g)}{mass oven dry (g)}
$$

Soil total organic carbon (SOC) was measured in all samples by dry combustion, using an Elemental analyzer (EAS 4010, Costech Analytical Technologies Inc., Valencia, CA). Soil organic matter was measured by loss-on ignition according to Nelson and Sommers (1996).

**Table 1.** General site description of the data set used to build the models. Mean values are of the collected samples (n=56, number of individual plots).




#### **2.3 Modelling and Statistical Analysis**

We developed multiple linear regression models by fitting the data using least squares by using bulk density (BD) as the response variable with either field collected data or data from the USDA WSS. Bulk density values were looked up on USDA WSS for each depth (0-15 cm, 15-30 cm, or 0-30 cm) at each sampling site. We used sand, clay, and silt percentage data from USDA WSS as factors in the model and saturation percentage as an independent variable. Soil texture values were looked up by depth for each site. We tested multiple linear regressions of different combinations of variables including OM, SOC, and texture. Total OM and SOC were measured in the lab from soil samples collected at each depth for each site, while texture was derived from USDA WSS. To test for different nonlinear regressions, we also included quadratic, cubic, and logarithmic terms for SP.

We randomized the order of the data set and trained the models using 80% of the data. Model predictions were tested with the remaining 20% of the data. Bagnall et al., 2021 suggested that experimental units should be excluded if the mean BD was greater than 1.8 g/cm<sup>3</sup> and SOC concentrations greater than 4.65%. However, none of the samples in our project were above these thresholds. We expanded the model generated by Abdelbaki et al. 2018 to evaluate additional models by including SP as one of the covariates. We compared the models using the adjusted  $R^2$  and Akaike's coefficient (AIC) to evaluate the

influence of the covariates in our models. Models with the highest adjusted  $R^2$  and lowest AIC, which represent the most precise and simple, are presented in this paper. We compared the estimated BD predicted by each model against the real measured BD data collected at each site. We calculated the root mean square error (RMSE) and  $R^2$  of the predicted values vs. the real values to compare each model's accuracy. R statistical software (R Development Core Team, 2021) and  $\alpha$  = 0.05 were used for all statistical analyses.

### **3. RESULTS AND DISCUSSION**

 Different models were fitted to the data, including different co-variates like texture (sand, clay, and silt percentages) and/or OM. The adjusted  $R^2$  and AIC values are listed in Table 1. Based on those parameters, the best models that predict BD for our data set were the linear model that relates BD (g/cm<sup>3</sup>) to SP (%) and OM (%) [1], the cubic model that relates SP and OM [2] and, the exponential model that relates OM and SP [3]. Polynomial model fits can be found in Figure 1.

 $BD = 0.008123 SP - 0.086929 \text{ OM} + 1.2248$  [1]  $BD = -6.115x10^{-6} SP^3 + 3.289x10^{-4}SP^2 + 2.813x10^{-2}SP - 0.07263 OM + 0.1777$ [2]

$$
BD = 1.67 e^{-0.0910C - 0.0024 SP} \quad [3]
$$

 The residual distribution for each model, shown in Figure 4, indicates that by including SP several times in the cubic model (Figure 4B), the variability of the predictions, measured by the size of the boxes, is reduced compared to the linear model (Figure 4A). The variability is also smaller for samples at 15-30 cm since soil at this depth is less subjected to disturbance effects from routine field operations.

 The predicted BD of each sample was calculated by inputting the measured values of SP and OM in our models. Model accuracy was tested by comparing the estimated values and the field BD. The results are shown in Figure 3 for all models, including the BD data from the USDA WSS. The predicted values were evenly distributed around the X=Y line, which means our three models are good at predicting BD (Figure 3A, 3B, 3D). The RMSE analysis shows that the exponential model (RMSE=0.028) is more accurate than the cubic model (RMSE=0.328). However, based on the  $R^2$  of the graphed values for field vs estimated BD (Figure 3B and 3D), the cubic model may be better suited for estimating BD with greater precision, since the estimated values have a better fit to the function  $X=Y$ , giving a higher  $R^2$ (0.395), compared to the exponential model ( $R^2$ =0.002).



**Figure 2.** Model fit for data collected combining 0-15 cm and 15-30 cm depth for the models in bold in Table 2. A) Linear Fit B) Cubic Fit. Fits generated with "geom\_smooth" function in R.

 An accurate estimation of BD is important to quantify changes in SOC and chemical soil properties. While previous work has correlated or attempted to predict soil BD to other edaphic actors like soil texture, SOC, and OM (Chaudhari et al., 2013; Sakin et al., 2011), a strong correlation has not always been apparent between BD and other edaphic factors.

Some researchers have demonstrated a strong relationship between BD and SOC (Tranter et al., 2007; Alexander, 1980; Abdelbaki, 2018). However, their  $R^2$  values have not been above 0.5 in most cases. For example, Abdelbaki's (2018) exponential model, as presented in Table 2, demonstrated a low RMSE (RMSE=0.105), indicating a high level of accuracy. When we incorporated SP into the equation evaluated by this previous research, it resulted in a decrease in RMSE (RMSE=0.028). Thus, including SP further improved the accuracy of the model. As SP accounts for available pore space in soil, it seemed likely that this measurement could be used to improve the model for predicting BD. Abdelbaki (2018) included a range of soil types and regions in their work, indicating that our model may be well suited to be expanded for additional growing regions beyond California.

**Table 2.** Adjusted R-squared and AIC values for different model fits. The three bold models are shown in Eq. [1], [2], and [3].





 BD is inversely proportional to soil porosity. Our model indirectly accounts for soil porosity by considering SP and OM content. SP serves as a proxy for micropore space, that is not disturbed after sieving. OM is related to macropore space associated with soil aggregation. Our models aim to capture a comprehensive picture of soil porosity, by indirectly considering both micropore and macropore spaces and their impact on bulk density.

 Unlike BD, which measures intact soil cores, SP is measured on sieved soil. While sieving soil will disrupt soil structure, the micropore space is not significantly disrupted and still reflects managed soils in California cropping systems, where frequent tillage (0-20 cm) is common to maintain short crop rotations. Bulk density values in USDA WSS, on the other hand, may not account for differences in agricultural field operations as compared to undisturbed soil, which justifies the high difference between BD values reported by USDA WSS and real BD values measured in the field (RMSE=1.38, Figure 3C). Furthermore, collecting BD samples in heavily disturbed agricultural soils can be challenging, particularly in the top 30 cm, due to loose soil or the presence of rocks and plant debris, including roots. One concern with sieving soil samples to measure SP is regarding more compacted soils. However, there is no apparent difference in the accuracy of the model for more moderately compacted soils (BD  $> 1.45$ ) as compared to less compacted soils (BD  $< 1.45$ ) in our models (Figure 4). The precision and accuracy of model estimations can change for highly compacted soils, with BD values outside of our data range (BD< 1.52), especially in the 15- 30 cm.

Saturation percentage has been correlated to physical soil properties. For example, Grewal et al. (1990) researched SP's applicability as an estimator of soil water measurements, specifically field capacity and wilting point. However, the linear relationship did not hold for available water capacity, because its highly affected by influenced by

109

weather-related factors. Our research included multiple soil chemical properties in various models to develop the best predictive model. In this case, a cubic model with SP and OM was strongest for predicting BD, with the highest precision ( $R^2$ =0.40, Figure 3A) and good accuracy (RSME=0.32, Figure 3A), while being the most efficient model (AIC= -87, Table 2).



**Figure 3.** Comparison of field bulk density measurements (Field BD) to model predictions (Estimated BD) for the A) Linear Model B) Cubic Model C) USDA WSS Bulk Density data, and D) Exponential Model. Models used are highlighted in bold in Table 2 and predictions are made with the test data set. Dotted line represents X=Y function.



**Figure 4.** Residuals of the comparison between field bulk density measurements (Field BD) to model predictions (Estimated BD) based on depth for the A) Linear Model B) Cubic Model C) USDA WSS Bulk Density Data base, and D) Exponential Model. A),B) and D) models are highlighted in Table 2.

 A pedotransfer function was developed from 124 long-term research sites in the United States, Canada, and Mexico, that successfully correlates plant-available waterholding capacity to soil calcareousness and SOC (Bagnall et al., 2021). They improved upon past models that demonstrated a negligible effect in changes in SOC on plant available water holding capacity. While our model includes only data from California's Central Valley, the Bagnall paper and Abdelabki (2018) demonstrate that models that document a strong relationship between soil chemical and soil physical properties can be successfully developed for wider geographic areas with sufficient data points. This is in contrast to conclusions drawn by other researchers, which have suggested that for the greatest precision, a correlative relationship between OM and BD should be derived from individual research sites and not on a larger scale (Harrison & Bocock, 1981).



**Figure 5.** Comparison of field bulk density measurements (Field BD) to model estimation of BD (estimated BD) for the A) Linear Model B) Cubic Model, and C) Exponential Model based on site, using the complete data set. Dotted line represents X=Y function.

## **Conclusion**

In this paper, we have demonstrated that the relationship between OM, SP, and BD exists and that it is a strong relationship. In the context of our findings, it is important to highlight that the predicted bulk density values generated by our model are most reliably suited for the 0-30 cm depth in tilled systems and may not accurately represent bulk density across all soil depths. It is important to point out that in heavily tilled systems, the 15-30 cm soil depth can be compacted; therefore, taking an undisturbed soil measurement is recommended, as the generated model does not account for compaction. However, with more samples, the models may be improved or adjusted to account for differences between high and low organic matter soils or other edaphic factors, like texture, which may impact the relationship between saturation percentage, soil carbon, and bulk density. We believe this model can be used in the Central Valley of California, but expanding the data set may allow for this relationship to be expanded and refined to be relevant for other regions.

The ability to quantify actual BD may be increasingly important as there is an interest in expanding carbon markets to include changes in soil carbon based on management practice. The quantification of actual changes in the volume of SOC requires BD, thus, this work is timely both for California and other regions. In addition, as we have demonstrated that predicted BD using our model is more accurate than USDA WSS values. Thus, our model appears to better respond to modifications to soil structure by standard agricultural field operations.

113

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# **Appendix**







