Title
Organic Matter Amendments Improve Soil Function in California Agroecosystems

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Organic Matter Amendments Improve Soil Function in California Agroecosystems

A dissertation submitted in partial satisfaction of the requirements for the degree of
Doctor of Philosophy

in

Environmental Systems

by

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I made it happen, now onward, and continuing to "make it happen."

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Villa, Y.B. and R.Ryals. (*July 2021*). Carbon sequestration potential in soils amended with biosolids is determined by site conditions and deep soil accounting. *(*Carbon Management, January 4, 2021). **will send upon request*

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Villa, Y.B. and R. Ryals. (2021). Quantifying Carbon Sequestration with Biosolids in CA. Biofest. Online. (Invited Oral).

Villa, Y.B. and R. Ryals. (2020). Long-term Application of Biosolids Increases Soil Carbon in Agricultural Soils. Soil Science Society of America. Online. (Oral).

Villa, Y.B. and R. Ryals. (2020). Long-term application of biosolids increases soil carbon in agricultural soils. California Plant & Soil Conference. Fresno, CA. (Poster).

Ryals, R. and **Y.B. Villa.** (2019). Quantifying the Climate Benefits of Biosolids Application to Agricultural Soils. American Geophysical Union. San Francisco, CA. (Poster).

Villa, Y.B. and R. Ryals. (2019). Carbon Sequestration from Biosolids Land Application. Webinar hosted by WEFTEC GHG Subcommittee. (Invited Oral).

Villa, Y.B. and R.Ryals. (2019). California Association of Sanitation Agencies CWEA Innovative Technology Seminar. Carbon Sequestration from Biosolids Land Application. Los Angeles, CA. (Invited Oral).

Villa, Y.B., S.D. Khalsa, R. Ryals, R. Duncan, and S.C. Hart. (2019). Soil quality response to organic amendments in almond orchards. Soil Science Society of America Conference. San Diego, CA. (Oral).

Ryals, R., **Y.B. Villa,** Kate Porterfield, Susan Cousineau, Fatima Gamiño, Teamrat Ghezzehei. (2018). Exploring the Beneficial Role of Biosolids in Soil Health and Climate Change Mitigation in California's Agricultural Soils. Bay Area Biosolids Coalition. (Oral).

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- Networked with other underrepresented professors from other HSI universities.

Abstract of the Dissertation

Organic Matter Amendments Improve Soil Function in California Agroecosystems

By

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Dr. Rebecca Ryals, Chair

Loss of organic matter (OM) from soil through degradation can lead to decline in soil structure, depletion of plant nutrients, enhancing mineralization of organic compounds and contribute to climate change through the release of greenhouse gas emissions. To address this, it is important to identify and expand our current understanding of the management practices that ensure that the functions and services of soils in agricultural lands are maintained for the long-term. Sustainable agriculture aims to meet society's needs for food, feed, and fiber, while protecting natural resources and is primarily guided by the quality of and aims to maintain or improve health of soils. A management practice that directly benefits soil quality, through the enhancement of soil organic matter (SOM), is the use of organic matter amendments (OMA). OMAs are a group of organic materials that are rich in nutrients that can be recycled back to the soil and can have positive effects for improving soil conditions and properties, and are proposed as a climate change mitigation strategy. Through extensive field, laboratory studies, and advanced analytical techniques, I show the potential benefits of recycling organic matter amendments into agricultural soils in California. Three projects were established and make up the three chapters of my dissertation: the first project focused on how the application of two different types of composts in almond orchards influenced soil fertility and nutrient cycling. Main findings from this chapter show that the applications of these OMA were heavily influenced by soil texture. After two years of OMA application there was an improvement in soil fertility. The second chapter focused on how long-term application of biosolids in agricultural soils influences carbon content, specifically focusing on the importance of accounting for deep soil carbon in order to determine a soils climate change mitigation potential in three sites in northern California. Main findings show that application rate of biosolids is not a determining factor when it comes to carbon accounting, but management practices play a bigger role. Additionally, not accounting for deep soil carbon leads to an underestimation of carbon sequestration in biosolid amended soils. Chapter 3 builds off Chapter 2, and investigates the stability of this C and N by determining what pools in the soil they are in. These different pools will provide insight on how vulnerable these reservoirs are to

management induced changes which can contribute to C and N losses. Overall findings show that long-term amended soils contribute increase carbon content associated to unprotected pools in surface soils where it is easier for microbes to mineralize. Carbon content in deeper soils show that the carbon is associated to physically protected and mineral associated pools, indicating this carbon to be more persistent. Overall, these findings show that site conditions, measurement methodology, and management practices influence the potential of OMA to be beneficial to soil fertility and climate beneficial.

1 Introduction

Agriculture and associated land-use change remains a major source of all three greenhouse gases (GHG): carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Paustian et al., 2016). Land-use contributed to ~25% of total anthropogenic GHG emissions globally: 10-14% from agricultural production, mainly via GHG via soils and livestock management, and another 12-17% from land cover change including deforestation (Paustian et al., 2016; Tubiello et al., 2015). Consequently, many government jurisdictions, in partnership with the 2015 Conference of the Parties to the UN Framework Convention on Climate Change (UNFCCC), have committed to reducing emissions across natural and agricultural lands as part of the "4 per mil" initiative, which aims to increase soil carbon (C) concentrations in agricultural soils globally by 0.4% per year (Grassi et al., 2017; Lal, 2016; Paustian et al., 2016; Roe et al., 2021, 2019). Hence, soil C sequestration has been proposed as one of the few strategies that could be applied at large scales and at a relatively low cost, compared to other engineered climate change mitigation strategies (Griscom et al., 2017; Roe et al., 2021). Although soils contribute to a large proportion of agricultural emission, improving their management can significantly reduce these emissions and sequester some of the CO_2 from the atmosphere via photosynthesis, and when remains of the photosynthetic green plants and other organisms enters the soil upon their death and becomes soil organic matter (Cameron et al., 2017; Roe et al., 2021).

Improved soil management not only sequesters soil C and contributes to decreasing GHG emissions, but efficient management can boost nitrogen (N) cycling, improve soil fertility, increase soil biodiversity, reduce erosion, runoff and water pollution, and can increase the resilience of agroecosystems (Lal et al., 2011; Paustian et al., 2016). Sustainable management practices that boost N availability, such as the applications of organic matter amendments like green waste, manure, or biosolids, enhance the buildup of soil organic matter (Christopher and Lal, 2007). Thus, increasing terrestrial C sequestration as a climate change mitigation strategy is considered a "win-win" because of it's indirect effect of improving soil fertility and productive capacity (del Mar Montiel-Rozas et al., 2016; Lal et al., 2003). Soil C sequestration is dependent on the availability of nutrients such as N. The additions of N through amendment application are necessary for increasing plant available N, which increases plant productivity, followed by increased C additions to soil through plant residues (Christopher and Lal, 2007; Ryals et al., 2015, 2014). Therefore, the utilization of organic amendments like composts and biosolids are proposed as one strategy to augment soil C sequestration and improve soil fertility.

The use of organic matter amendments is not only beneficial on a soil fertility and soil C sequestration perspective, but also the recycling of these nutrients has implications on closing nutrient loops. Typically, organic matter amendments are in the form of yard wastes, manure, and human waste, are diverted into landfills where they may contribute to GHG emissions and groundwater contamination (Borjesson and Katterer, 2018; Brown et al., 2011; Harrison et al., 2020; McNicol et al., 2020). Broken nutrient and carbon cycles in food and urban systems waste large quantities of organic resources which contributes to climate change, food insecurity, and soil degradation (Ryals et al., 2019). Closing these cycles may contribute to reducing GHG goals, improve the resiliency of agroecosystems, and contribute to overall societal sustainability goals (Lal et al., 2011; Ryals et al., 2019; Schipanski et al., 2016).

Due to all these benefits of organic matter amendments in agricultural soils, it is important to understand how to properly manage these materials to enhance their potential for improving soil fertility and climate change mitigation potential (Villa et al., 2021; Villa and Ryals, 2021). The use of these organic matter amendments vary across agroecological contexts, such as organic amendment type, soil texture, type of cropping systems, water management, nutrient management, and how the amendment is applied influences its efficacy. The overall goal of my dissertation is to investigate how organic matter amendments affect soil fertility and C sequestration in soils across different agroecological contexts.

Chapter 1 focuses on assessing how two compost types, green waste and composted manure, contribute to soil fertility in almond orchards with contrasting soil textures during a two-year time period. Inorganic nitrogen (N) increased the month following OMA application; but was short-lived. Concentrations of inorganic N decreased down to preapplication levels suggesting that N was lost through leaching, mineralization, or taken up by plants. The orchard with coarser soil texture did not experience an increase in cation exchange capacity (CEC) or available potassium (K) after two-years of amendment application; whereas; the orchard with the finer soil texture increased in both significantly. Both orchards increased significantly in total carbon (C) and N, however, the magnitude of the increase was higher in the coarser soil textured orchard relative to the control (Villa et al., 2021).

Chapter 2 investigates how long-term application of biosolids, treated organic waste from treatment plants, effects C storage down to one meter depths in three agricultural sites in northern California. Each site varied in conditions and management practice which we found to significantly influence how C and N was stored. One site had biosolids applied yearly and had fine soil texture; however, we found that the highest concentrations of C and N was found in the top 10 cm of soil, no significant changes were observed down to one meter depths. We speculated that flood irrigation possibly affected the stability of the C and N added from the biosolids, causing them to mineralize and be lost from the soil system. The second site had a moderate amount of biosolids application (every three years for 20-years), had fine soil texture like the first site, was rainfed irrigated, and had the most significant amount of C and N increases down to one meter depths. The last site had the lowest amount of biosolids application (every five years for 20 years), coarser soil texture, was flood-irrigated, and experienced no significant increases in C and N down to one meter depths. However, data shows that there is slight increases in amended soil in deeper depths, though not significant, it still shows how C and N can move through the soil profile. Additionally, we compared the rate of C sequestration using three approaches: 1) an areal approach from 0-30 cm; 2) an areal approach taking into account deep soil down to one meter; 3) amount of C stored per amount of biosolids applied. Our goal was to determine if there would be an underestimation if deep soil C was not taken into account. Our most interesting result was that only taking the top 30 cm into account in the last site with coarser soil texture would show that C is being lost, but C is being sequestered when deep soil C accounted for (Villa and Ryals, 2021).

Chapter 3 builds off Chapter 2, and investigates the stability of this C and N at these three sites by determining what pools in the soil they are in. These different pools will provide insight on how vulnerable these reservoirs are to management induced changes which can contribute to C and N losses. Further investigations on stabilization mechanisms show that in the sites with finer texture and lower pH have SOC is associated in organo-mineral complexes. Calcium and SOC correlations in one of the sites show that soils with biosolids application had weaker correlations than control soils indicating SOC is preferentially binding to biosolids instead of calcium. Overall, these findings show that site conditions, measurement methodology, and management practices influence the potential of OMA to be beneficial to soil fertility and climate beneficial.

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2 Organic Matter Amendments Improve Soil Quality in Almond Orchards with Contrasting Soil Texture

2.1 Abstract

The effects of organic matter amendments (OMA) on soil fertility in permanent cropping systems like orchards is under-studied compared to annual cropping systems. We evaluated experimentally the impact of OMAs on soil fertility in almond (*Prunus dulcis*) orchards over a two-year period with annual applications. Two OMAs, derived from composted green waste (GWC) or composted manure wood chips (MWC), were applied as surface mulch and compared to a control at two sites with different soil textures (sandy loam and loamy sand). OMAs increased soil moisture content (0-0.1m depth) at both sites by 27%-37%. Both amendments increased soil inorganic N at the sandy loam (GWC: 194%; MWC: 114%) and loamy sand (GWC: 277%; MWC: 114%) sites the month following application, but soil inorganic N concentrations quickly decreased to values similar to those of control plots. After two-years, the GWC and the MWC amendments increased the soil cation exchange capacity (CEC) by 112% and 29%, respectively, in the sandy loam site, but no change was observed in the loamy sand site. The greatest increase in soil extractable K occurred in the GWC-amended plots at the sandy loam site even though the initial K concentration of MWC was higher. Both OMAs increased soil organic carbon (SOC) after two years, but the SOC increase in the GWC-amended plots was greater. Our results suggest that OMAs can significantly improve soil fertility after one or two annual applications, and that fertility gains appear to be dependent on soil texture than the nutrient concentrations of the OMA.

Keywords: cation exchange capacity, composted manure, decomposition, green waste, nutrient dynamics, perennial crops

2.2 Introduction

Abundant sources of organic matter in the Central Valley, such as green wastes from agriculture and urban areas and manures from dairy production, can help augment soil organic matter (SOM) levels in permanent crops like almond orchards (Khalsa and Brown, 2017). Typically, SOM levels in California's Central Valley soils range from 0.5% to 2.5% (Warren, 1996). Permanent crops like almonds planted on 562,525 ha are a prime candidate for the application of organic matter amendments (OMAs). In this region, 82% of the world's almonds are produced (California Almond Board, 2018). Although almond trees generally grow best in sandy soils, expansion of almond orchard into soil types with higher silt and clay content has become more common. A poor understanding of how to manage OMAs in orchards limits their use and weakens the potential for improvement in soil fertility in these agroecosystems (Khalsa and Brown 2017). Although there have been many studies that have focused on the effects of OMAs in row crops and permanent crops in other regions, few have focused on how these amendments affect soil fertility in permanent crops in California (Baldi et al. 2018; Blackshaw et al. 2005; Canali et al. 2004; Forge et al. 2013; Gannett et al. 2019; Lepsch et al. 2019; Peck et al. 2011). Understanding how surficial applications of OMAs influence nutrient dynamics in the short-term in permanent cropping systems poses different challenges than in annual cropping systems where OMAs are incorporated directly into the soil (Jackson et al., 2003).

Farmers and growers use many practices that promote soil fertility, minimize water use, and lower nutrient losses from the field. An example of this is the capture, conversion, and reuse of organic wastes as OMAs to agricultural soils. Sources of organic wastes include livestock manures, food waste, yard debris, and biosolids. These materials are rich in nutrients and organic matter that could be recycled back to the soil, improve soil fertility, and minimize environmental consequences of landfilling or other waste management fates. Composting organic wastes prior to land application slows the rate of decomposition and nutrient release compared to the organic wastes in their raw, undecomposed form (Hue and Liu 1995). There is increased opportunity to recover and reuse organic wastes by applying these materials to agricultural soils as composted OMAs due to new policy incentives aimed at improving soil fertility and enhancing soil carbon (C) sequestration. For example, the state of California recently passed several pieces of legislation aimed at diverting organics from landfills and increasing composting (e.g., SB 1383, AB 1594, Healthy Soils). Improper disposal of these organic wastes can cause detrimental environmental effects such as increased greenhouse gas emissions and water and air pollution (Borjesson and Katterer 2018; Brown et al. 2011). Composting can minimize these negative impacts and also build productive soils through the introduction of exogenous organic matter.

Composts can be comprised of different organic materials that may also experience diverse composting processes that affect their physical state (e.g., particle size) and chemical composition. Composting involves biological decomposition that transform heterogeneous organic waste under relatively controlled environmental conditions of moisture, temperature, and aeration to more homogeneous organic matter (Acatrinei et al. 2019). Variations of these conditions change the end product of the composted OMAs, which influences the subsequent decomposition of the applied material and their impact on soils processes (Diacono and Montemurro 2010). Additionally, the carbon to nitrogen (C:N) ratio of the initial and final organic material influences the persistence of the OMA and subsequent release of nutrients available for plant uptake (Hue and Liu 1995; Diacono and Montemurro 2010; Feng et al. 2013; Leon et al. 2015; Liu and Zhou 2017; Nicholson et al. 2018).

Soil texture may mediate the impacts of OMAs on soil fertility. Soils with a higher clay fraction tend to have a greater proportion of micropores to store plant-available water than soils with coarser soil texture. The greater specific surface area and negative charge density of clay-sized soil separates also lead to greater capacity for nutrient storage (Sarker et al. 2018). Furthermore, soils with higher clay content tend to exhibit higher SOM persistence due to the physical interactions of these particles with SOM (Paul 2016; Torn et al. 1997). Hence, soils with higher clay content are typically higher in SOM content, store greater amounts of plant available water and nutrients, and are less susceptible to nutrient loss via leaching than soils with coarser textures (Diacono and Montemurro 2010; Ding et al. 2014; Sarker et al. 2018). In coarser textured soils, OMAs applied to row crops have been shown to improve soil chemical and physical properties, such as increases in soil water holding capacity, aggregate stability, and cation exchange capacity (CEC; Ozores-Hampton et al. 2011). Application of OMAs to finer textured soils may increase the relative abundance of macropores, improving aeration, water infiltration, and soil aggregation. Regardless of soil texture, the resultant changes in soil pore size distribution from OMA addition typically lead to greater plant available soil water storage (Eusufzai and Fujii 2012).

We determined how the application of two different OMAs influences soil fertility in two almond orchards with contrasting textures. We focused on soil measurements in our study because the almond tree were not of fruit bearing age. We hypothesized that: 1) the manure wood chip compost (MWC) will decompose and release nutrients to the soil more slowly than green waste compost (GWC) because of the higher initial C:N ratio of MWC; 2) OMA additions will improve soil moisture content because organic matter increases water holding capacity by improving soil physical properties; 3) the application of both OMAs would increase total C, total nitrogen (N), extractable potassium (K), and plant-available N and phosphorus (P) in both soils; and 4) OMA application to coarser textured soil (loamy sand) would increase the soil CEC to a greater relative extent than the finer textured (sandy loam) soil because of the former having an inherently lower CEC. We evaluated these hypotheses in two almond orchards of contrasting textures that received annual applications of GWC and MWC over a two-year period.

2.3 Materials and Methods

2.3.1 Study sites

We conducted this study in two almond orchards approximately 8 km from each other near Modesto, California (37.71 °N, -120.95 °W and 37.73 °N, -121.01°W; 27 m above sea level), over two growing seasons (February 2016 through February 2018). Soils in the region are developed from granitic alluvium (Storie and Weir 1953). Soils in the first orchard are classified as fine, smectitic, thermic Abruptic Durixeralfs USDA Soil Taxonomic family, and have a sandy loam surface soil texture (in the 0 – 0.5 m soil depth (mean ± standard error, n = 4): 57 ± 1.1% sand, 33 ± 1.1% silt, 10 ± 0.1% clay), determined using the hydrometer method as described in Gee and Bauder (1986). Soil pH was determined to be 7.37 ± 0.05 measured in an 1:2 soil to deionized water (w/v) suspension (McLean, 1982) for the 0 – 0.5 m soil depth (mean ± standard error, n = 4). This orchard will be referred to hereafter as the “Loam site.” The soils in the second orchard are classified as mixed, thermic Typic Xeropsamments, and have a loamy sand surface (0-0.5 m) soil texture (89 ± 0.2% sand, 7 ± 0.4% silt, 4 ± 0.4% clay) with a pH of 7.82 ± 0.06. This orchard will be referred to hereafter as the “Sand site.” Mean annual precipitation in this region (failing entirely as rain) is 335 mm and mean annual air temperature is 17.5 °C (U.S. Climate Data, 2018).

2.3.2 Experimental design

The almond trees in the Loam orchard were the cultivar 'Nonpareil' and in the Sand site the trees were the cultivar 'Independence'. The current trees occupying both orchards were planted in 2013 and at the time of the study, were not producing fruit. Soils were tilled before tree planting. Experimental controls never received any OMA, only inorganic fertilizers. Immediately prior to establishment of the current almond orchards, the Loam site was a fallowed field and the Sand site was an older almond orchard. At the beginning of this study, trees were approximately 1.5 m tall with a tree trunk circumference at breast height (1.4 m) of 0.5 m. Both almond orchards are irrigated using micro-sprinklers at a rate of 20,860 mm yr^{-1} and were fertilized with urea ammonium nitrate (UAN) 32% N at a rate of 139 kg N ha^{-1} yr^{-1} in 2016, and 207 kg N ha^{-1} yr^{-1} in 2017. At each site, fertilizer was applied via irrigation three times during the growing season for both years: in late February before compost application, in mid-June, and late July. OMAs were applied to the soil surface in a 1.2 m band at 11,570 kg ha^{-1} (Loam site) or 18,160 kg ha^{-1} (Sand site) prior to planting. OMAs were incorporated into the planting berm when trees were machine-planted in January 2013. Subsequently, OMAs were placed on the soil surface near each tree (1,230 kg ha^{-1} at both sites) in May 2015. Both OMAs were applied again to the soil surface in late February-early March in 2016 (28,020 kg ha^{-1}) and 2017 (26,900 kg ha^{-1}), concentrated in a 3 m band down the tree rows.

Experimental treatments included two types of OMA, manure wood chip compost (MWC) and green waste compost (GWC), as well as an unamended control. Although the two OMAs each year were obtained from the same suppliers (GWC: Recology Premium Compost® from Recology Organics, Vernalis, CA, USA; MWC: product from Epic Soils, Oakdale, CA, USA), the initial elemental composition of the same OMA type applied differed between years (Table 1). Treatment plots were established in the almond orchards using a randomized block design (n = 4 at the Loam site and n = 5 at the Sand site; Supplemental Figure 2). In the loam site, each treatment plot consisted of 12 almond trees in a row; trees were spaced 4.9 m apart within the row and rows were planted 6.7 m apart. At the Sand site, each block was 15 trees long; trees were spaced 4.3 m apart and rows were 6.4 m apart. The Loam site planted with 'Nonpareil' had four plots, separated by a row of pollinizer cultivars 'Wood Colony' and 'Aldrich'. Plots in the Sand site planted with 'Independence' were adjacent to each other as no pollinizer is required for this self-fertile variety (Supplemental Figure 2). Hobo soil thermometers were deployed at 0.1 m from the soil surface (n = 9 each site, total n = 18). Each treatment had three thermometers per site for replication. Measurements were programed to record soil temperature every hour from the Spring of 2017 to Spring of 2018.

2.3.3 Decomposition rates and nutrient analysis of organic matter amendments

Initial compost samples from 2016 and 2017 were shipped to the UC Davis Analytical Laboratory for macro- and micro-nutrient analyses. Subsamples were digested using nitric acid/hydrogen peroxide microwave digestion and then analyzed on an Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) for total P, K, sulfur (S), calcium (Ca), magnesium (Mg), iron (Fe), boron (B), copper (Cu), manganese (Mn), and zinc (Zn; Meyer and Keliher, 1992; Sah and Miller, 1992).

In both years, decomposition rates and nutrient release from MWC and GWC amendments were assessed using decomposition rings that consisted of 0.2-m tall, 0.1- m diameter PVC pipe, with a mesh screen (0.794 mm openings) attached to the bottom. Each decomposition ring was filled with 50 g fresh weight of either MWC or GWC to simulate field application and rate applied in the Spring of 2016 and 2017. Twenty-four rings were placed randomly within the rows, at least 1 m away from trees, in each MWC and GWC treatment plot, and secured to the ground using rebar tie wire. Some decomposition rings were physically lost (~12), while the data from some other decomposition rings were not used because of substantial contamination by mineral soil or unrealistic disappearance of organic matter from the decomposition ring (presumably due to disturbance by wind, animal activity, or agricultural machinery). Decomposition rings were also placed on top of each of the soil cores (0 – 0.5 m) in the MWC and GWC treatment plots (see Supplemental Figure 1). Two decomposition rings were randomly collected (n = 8 at the Loam site, n = 10 at the Sand site) and composited from each plot at approximately 30-day intervals during the two growing seasons. The samples were place on Rubbermaid Blue Ice® in a cooler, then transported back to the laboratory at the University of California (UC) Merced for gravimetric water content and chemical analyses. Although we originally planned to conclude this component of our study at twelve months, substantial mineral soil contamination occurred in most of the decomposition rings after nine months following the application of the OMAs, or all of the compost within a decomposition ring had disappeared presumably due to disturbance as noted above. Decomposition rings in the second year of this study had significant mineral contamination after only 4 months. Hence, we only used data collected from the first nine months during year one for determining C, N, P, and K loss during OMA decomposition.

Field-collected MWC and GWC from decomposition rings were weighed (field-moist) within 24 h after collection. Approximately, 15-g fresh-weight subsamples were placed in a 105 °C oven for 24 h to determine gravimetric water content and to calculate the dry-mass remaining in the ring. Air-dried GWC and MWC samples were coarsely ground through a 1-mm mesh screen using a large Thomas Wiley Model 4 Mill (Swedesboro, NJ, USA). Subsamples (~4 g) of these materials were then finely ground through a 420 μm (40 mesh) screen using a Wiley Mini Mill. Finely ground samples were analyzed for total C and N-Combustion on a TruSpec CN Analyzer at UC Davis Analytical Laboratory.

Decomposition rates of OMAs were calculated as the net decrease in C mass remaining over time. Similarly, the net release of N, P, and K from these materials during decomposition was estimated as the net decrease in the masses of these elements remaining in the OMA over time. Elemental masses were calculated by multiplying the oven-dry concentrations of the respective element by the oven-dry mass of OMA remaining.

2.3.4 Plant available soil nutrients

Plant available soil nutrients (nitrate, NO_3; ammonium, NH_4^+; and phosphate, PO_4^{3-}) were determined in situ using ion exchange resin (IER) bags placed at the bottom of intact soil cores (0.5-m in length) contained within solid 0.1-m diameter PVC pipes (a variation of the Resin-Core method where only the IER bags are analyzed; Binkley and Hart 1989; Supplemental Fig. 1). IER resin was placed at 0.5 m depth in order to capture nutrient availability in the active rooting zone, which has been determined to be from 0.2 – 0.6 m under micro-irrigation *(*Brown and Sanden *unpublished data*; Lepsch et al., 2019; Vrugt et al., 2001). Two PVC pipes were deployed within a treatment plot. Placement of the PVC pipes was determined by randomly selecting two locations within the row.

Resin-cores were harvested after one year (Feb. 2016 - Feb. 2017), and the process was repeated for a second year (Feb. 2017 - Feb. 2018). Harvested resin bags were transported in a cooler containing Blue Ice® for analysis at UC Merced. Resin beads were air-dried, mixed, and a subsample of 15 g was extracted with 50 ml of 2 M KCl. Resin suspensions were shaken for one h, then filtered with Whatman #1 paper that was pre-leached with deionized water. Filtered extracts were then stored at -20 °C until they were ready for analysis. Thawed extracts were mixed and then analyzed for NO_3^-, NH_4^+, and PO_4^{3-} concentrations on a Lachat QuikChem 8500 Flow Injection Analyzer (Loveland, CO, USA) using methods 12-107-04-1-B,12-107-06-1-B, and 10-115-01-1-A, respectively. Available N and P over the two years were calculated by summing the total inorganic N and PO_4^{3-}, respectively, extracted from the resins collected in year one and year two, and expressing these data on an areal basis (using the cross-sectional area of the IER bag; 0.0078 m^2).

2.3.5 Soil analysis

Mineral soils (0-0.1 m depth) were collected every other month using an Oakfield soil probe (Oakfield, WI, USA; 0.17-m diameter) to determine soil inorganic N pool sizes. Soils were collected at this depth because OMA application is surface applied as a mulch and it has been determined that the active rooting zone is from 0.2 – 0.6 m under micro-irrigation *(*Brown and Sanden *unpublished data*; Lepsch et al., 2019; Vrugt et al., 2001). Two soil cores were taken randomly within a treatment block, composited, and then transported back to the laboratory at UC Merced in a cooler containing Blue Ice®. Fresh soil was used to measure gravimetric soil moisture content (105 °C oven for 24 h). A subsample of 15 g was shaken with 50 ml of 2 M KCl for an hour then filtered using Whatman 1 filter paper. Soil extracts were frozen until ready for analysis on the Lachat QuikChem 8500 Flow Injection Analyzer (Loveland, CO, USA) using methods 12-107-04-1-B for NO_2/NO_3 and 12-107-06-1-A for NH_4, respectively. The remaining soil was air dried and used for fertility analyses.

Soils used for CEC and total organic C, N, and K were collected pre-application and after the second year of study. Sieved, air-dried soils were analyzed for cation exchange capacity (CEC) using the barium method (buffered to pH 7.0) at the UC Davis Analytical Laboratory (https://anlab.ucdavis.edu/analysis/Soils/430; Rible and Quick, 1960). Subsamples of air-dried were ground to a fine powder using a mortar and pestle and approximately 15 mg of ground soil was weighed into tin capsules for total C and N analysis (Costech ECS 4010 CHNS-O Elemental Analyzer coupled with a ThermoFisher Delta-V Plus continuous flow isotope ratio mass spectrometer) at the UC Merced Stable Isotope Laboratory (https://research.ucmerced.edu/core-facilities/stable-isotope-laboratory). Soils were corrected for air-dry moisture and no inorganic C was present as indicated by a lack of effervescence when 4 N HCl was added to soils.

2.3.6 Statistical analysis

Initial characteristics of OMAs used in both years were analyzed using a one-way ANOVA with amendment type as a factor in the model, and a Tukey's multiple comparisons post hoc test was employed when ANOVA models were significant. Net release of C (i.e., decomposition), N, P, and K were analyzed separately using a two-way repeated measures ANOVA where OMA and site were factors for the model. Interactions of soil NO_3^- and NH_4^+ from 0-0.1 m soil collected monthly were summed together to get total inorganic N, and were analyzed using a two-way repeated measures ANOVA to determine treatment and site effects through time. Additionally, total inorganic N data was split by site and analyzed using a two-way repeated measures ANOVA with treatment and month as factors. Available N and P from IER bags were analyzed using a three-way repeated measures ANOVA with treatment, site, and year as factors. In order to explore if there were significant changes in soil organic C, total N, extractable K, and CEC from pre-treatment (T = 0) and post-treatment (T = 2 yrs.), we employed a three-way repeated measures ANOVA with treatment, site, and year as factors for the model and interactions. Soil moisture and temperature were analyzed using a two-way repeated measures ANOVA with treatment and orchard as factors.

Statistical tests were performed using RStudio 3.3.1. All analyses were tested for normality using the Shapiro-Wilk test. Heteroscedasticity was analyzed by plotting residuals on a qq-plot. Variables that were not normally distributed were log transformed ($\log_{10}$) to meet assumptions for ANOVA. Arithmetic means are shown in tables and figures. Statistical significance was established a prior at $\alpha = 0.05$.

2.4 Results

2.4.1 OMA initial nutrient composition, decomposition, and nutrient dynamics

Initial OMAs nutrient composition was different between years for some elements (Table 1). The MWC in 2016 and 2017 had 60% higher total C concentrations ($p < 0.001$) and 64% higher C:N ratio ($p < 0.001$), and lower concentrations of P (61%; $p < 0.001$), S (24%; $p < 0.001$), Ca (19%; $p = 0.007$), Mg (8%; $p = 0.040$), Cu (35%; $p < 0.001$), Mn (31%; $p < 0.001$), and Zn (22%; $p = 0.004$) in 2016 compared to 2017. However, initial concentrations of N ($p = 0.954$), K ($p = 0.906$), Fe ($p = 0.782$), and B ($p = 0.173$) were similar between years within an OMA type. The initial nutrient concentrations of K (10%; $p = 0.009$), S (10%; $p = 0.033$), B (10%; $p = 0.020$), and Cu (15%; $p < 0.001$) in the GWC OMA were higher in 2016 than in 2017. However, the initial nutrient concentrations of C ($p = 0.831$), N ($p = 0.792$), C:N ratio ($p = 0.415$), P ($p =0.944$), Ca ($p = 0.897$), Fe ($p = 0.849$), Mg ($p = 0.614$), Mn ($p = 0.169$), and Zn ($p = 0.900$) in the GWC were similar between years.

We found that net loss of C ($p = 0.120$), N ($p = 0.059$), and P ($p <0.001$) was generally faster for MWC than GWC, but only net P release was statistically significant

(Fig. 1a-c). Net nutrient loss of these same nutrients was also significantly influenced by site, with net release generally faster in the Loam than the Sand site (C, $p = 0.002$; N, $p = 0.007$; and P, $p = 0.084$). Net K release during OMA decomposition was not influenced by OMA type ($p = 0.339$) or by site ($p = 0.686$; Fig. 2d). For all four nutrients, we found no significant interaction between OMA type and site ($p > 0.640$).

2.4.2 Soil moisture response to OMA

There were significant treatment ($p<0.001$) and site ($p<0.001$) effects on soil moisture (0 – 0.1 m depth), with no significant interaction between these two factors ($p = 0.340$; two-way repeated measures ANOVA). A two-way repeated measures ANOVA model for the Loam site showed soils amended with MWC ($p < 0.001$) and GWC ($p <0.001$) had a 29% and 29% higher soil moisture content than the control, respectively. No interaction was observed between treatment and month ($p = 0.872$). No differences were observed between both OMA at the Loam site ($p = 0.872$; Fig. 2). At the Sand site, soils amended with both MWC (28%) and GWC (37%) had higher soil moisture content than the unamended control soils (both $p < 0.001$). There was no interaction between treatment and month ($p = 0.466$). Soil moisture content did not differ significantly between the two OMAs ($p = 0.748$). Soil temperature (0.1-m depth) was not significantly influenced by OMAs ($p= 0.321$) or soil type ($p = 0.210$; data not shown; Loam site = 16.7 ± 0.1 °C; Sand site= 16.6 ± 0.1 °C).

2.4.3 Soil inorganic N response to OMA

A two-way repeated measures ANOVA was performed with treatment and site as factor showed no significant difference in soil NH_4^+ concentrations (0-0.1 m) among treatments during the two-year experiment ($p = 0.480$). No site ($p = 0.092$) or treatment by site interactions were observed ($p = 0.870$).

The application of OMAs significantly increased NO_3^- concentrations (0-0.1 m) in amended soils ($p<0.001$). Concentrations of NO_3^- were significantly different between the control and the MWC ($p=0.028$) and the GWC ($p=0.004$) treatments. There were also differences observed between the Loam and Sand site ($p<0.001$). No interactions were found between sites and treatments ($p=0.745$).

Total inorganic N concentrations were significantly different between the control and MWC ($p = 0.025$) and GWC ($p = 0.004$; Figure 3). No differences were observed between the OMA types ($p = 0.710$) and no interactions were observed between treatment and site ($p = 0.763$). We also found total inorganic N concentrations differed between the Loam and Sand sites over the two-year study (both $p < 0.001$). A two-way repeated measures ANOVA showed no significant differences in total inorganic N concentration among the treatments in the Loam site ($p = 0.216$). However, differences were observed among months ($p = 0.005$), with no interactions between month and treatment ($p = 0.909$). A two-way repeated measures ANOVA showed significant treatment differences ($p = 0.001$) in total inorganic N concentrations between the control and both MWC ($p=0.042$) and GWC ($p=0.012$) treatments at the Sand site. Both OMAs were not significantly different from each other in total inorganic N concentrations ($p = 0.771$). Total inorganic N concentrations were different among months at the Sand site ($p = 0.016$).

2.4.4 Available N and P measured by IER

Inorganic N fluxes measured by ion exchange resins were not significantly different among treatments ($p = 0.518$). However, inorganic N concentrations from IER showed differences between sites ($p < 0.001$) and year ($p = 0.046$). There was no interaction between treatment and year ($p = 0.713$), treatment and site ($p = 0.640$), and treatment, site, and year ($p = 0.667$; Fig. 4a.). A similar three-way repeated measures ANOVA model was used to determine differences between resin P concentrations. There were significant treatment ($p = 0.036$; Fig. 4b.) and site differences ($p < 0.001$). However, there was no difference between years ($p = 0.648$). A marginally significant interaction between treatment and year occurred ($p = 0.067$). No interaction was observed between treatment and site ($p = 0.245$), and treatment, year, and site ($p = 0.961$).

2.4.5 Soil organic C and total N

Soil organic C and total N concentrations (0 – 0.1 m) were analyzed prior to OMA application in 2016 and at the end of the study (2018). Results from the three-way repeated measures ANOVA showed treatment ($p < 0.001$), site ($p < 0.001$), and year ($p = 0.004$) effects on soil organic C, with a significant treatment by year interaction ($p = 0.002$). No significant interactions were observed between: treatment and site ($p = 0.306$); site and year ($p = 0.411$); and treatment, site, and year ($p = 0.912$). A Tukey's post hoc test shows soil organic C concentration significantly increased from pre- (2016) and post- (2018) application in both the MWC ($p = 0.006$) and GWC ($p = 0.006$) treatments, but no change was observed in the control ($p = 0.707$). The MWC treatment increased soil organic C by 33% in the Loam site and by 50% in the Sand site after two years of OMA application (Figure 5a). The GWC treatment increased soil organic C by 92% in the Loam site and 67% in the Sand site. Significant treatment, site, and year main effects occurred for soil total N concentration (all $p < 0.001$). A significant treatment by year interaction also occurred ($p = 0.002$). There were no significant interactions observed between: treatment and site ($p = 0.313$); site and year ($p = 0.439$); and treatment, site, and year ($p = 0.914$). A Tukey's post hoc test showed both the MWC ($p = 0.020$) and the GWC ($p = 0.001$) treatments increased soil total N concentrations from pre- and post- applications (i.e., after two years), while the control ($p = 0.970$) did not change (Figure 5b). Soil total N concentration increased in the MWC treatment by 36% in the Loam site and by 80% in the Sand site after two years of OMA applications. Soil total N concentration in the GWC treatment increased by 75% in the Loam site and by 100% at the Sand site from 2018 to 2019.

2.4.6 Soil K availability and CEC

Results showed K availability (as measured by extractable K in top 0.1 m soil) was significantly different among treatments, and between sites and years (all $p < 0.001$), with a significant site by year interaction ($p = 0.004$; Fig. 6b). No significant interactions were observed: between treatment and site ($p = 0.825$); treatment and year ($p = 0.174$); or treatment, site, and year ($p=0.102$). Soil available K in MWC plots increased by 14% in the Loam site and by 9% in the Sand site. A Tukey's post hoc showed that the MWC ($p < 0.001$) and GWC ($p < 0.001$) were significantly higher than the control, but both amendments were not significantly different from each other ($p = 0.950$). The GWC treatment contributed to a 138% increase in available K in the Loam site and a 7% decrease in the Sand site. The controls increased by 45% in the Loam site and by 11% in the Sand site.

Soils (0-0.1 m depth) collected from both sites before treatment and after two years of OMA application showed significant increases in CEC. Three-way repeated measures ANOVA showed significant treatment ($p < 0.001$), site ($p < 0.001$), and year ($p = 0.007$) effects (Fig. 6a). An interaction occurred between treatment and year ($p = 0.001$), showing differences in soil CEC between control and MWC ($p = 0.045$) and GWC ($p < 0.001$) in 2018. There was a marginally significant three-way interaction among treatment, site, and year ($p = 0.052$), and no significant interaction between treatment and site occurred ($p = 0.279$). Tukey's post-hoc test showed that the GWC treatment significantly increased in CEC from pre-application to post-application ($p < 0.001$), but the MWC treatment did not significantly increase CEC ($p = 0.780$). The CEC in the control remained similar after two years ($p = 1.000$). Soil CEC increased by 29% and 14% after two years of OMAs from pre- and post- application in the Loam and Sand sites, respectively. The greatest relative change in soil CEC occurred with the GWC treatment, where the soil CEC increased by 90% in the Loam site and by 25% in the Sand site (Fig. 7).

2.5. Discussion

Our study shows that the application of OMAs improves soil fertility (0-0.1 m) through the enhancement of soil water content, CEC, and total C and N. Although, increases in inorganic N were short-lived, continued application of OMAs may lead to more sustained increases in inorganic N. Moreover, the effect of OMAs on orchard soils depended on the soil type, suggesting that soil texture (and potentially other unmeasured soil properties) modulates the soil fertility response to OMAs. Results of this study demonstrated that application of OMAs can positively influence soil chemical properties and soil water dynamics of orchard soils in the short-term.

2.5.1 OMA decomposition rates and net nutrient release

Decomposition of organic matter represents the primary source of nutrients for plants, and nutrients and energy for soil microbes in unfertilized fields. We hypothesized that the decomposition rate (i.e., net decrease in C) and net nutrient release from the GWC would be higher than from the MWC because of the lower initial C:N mass ratio of the former OMA in the 2016 amendments (Table 1). However, in contrast to our hypothesis, we generally found faster decomposition and net release of N and P from the MWC amendment than from the GWC amendment. Faster decomposition and net N and P release from the higher initial C:N ratio amendment could be due to the finer particle size of the MWC than the GWC amendment, which would result in both greater surface area for microbial colonization in the MWC and greater ease of movement of this material out of our decomposition rings and into the mineral soil (Ozores-Hampton et al., 2011). Alternatively, these differences in decomposition rate and net N and P release may also be due to unmeasured differences in the water-soluble components between the two amendments, with higher soluble fractions in the MWC than the GWC (Dey et al., 2019).

Interestingly, even though the OMAs were applied to the soil surface and not incorporated into the mineral soil, the decomposition rate and net N and P release was higher in the loam site than the sand site. Because soil temperatures and exogenous water and inorganic nutrient inputs were similar between sites, this result suggesting that the underlying mineral soil texture influenced these nutrient cycling processes (Forte et al. 2017). Higher rates in the loam site could be the result of greater underlying soil moisture in the loam site than in the Sand site, increasing the availability of water to microbial decomposers in the OMAs. Capillary fringe movement of water from the mineral soil to the overlying OMAs would be expected to be greater in the loam than the Sand site soil (Weil and Brady, 2017).

As has been observed in other decomposition studies using fresh plant litter (Harmon et al., 2009; Hart et al., 1992) or composted organic matter (Bar-Tal et al., 2004; Wang et al., 2015), net release of K from the OMAs in this study was much faster than C, N or P loss, suggesting that K release is governed primarily by physical rather than biological processes (Bar-Tal et al., 2004). Similarly, a study focusing on OMA application in almond orchards by Khalsa and collaborators (unpublished data) found that K nutrient release was more rapid than C, N, and P. In support of this hypothesis is our finding that OMA type had no significant effect on net K release despite the higher initial K concentration of MWC than GWC (Table 1). Additionally, unlike the more microbial-influenced nutrients N and P where site was a significant factor, net K release was unaffected by site. Taken together, these results support other OMA decomposition studies that demonstrate that, unlike fresh plant litter, decomposition and net nutrient release from OMAs are not strongly regulated by their initial chemical composition (Ryals and Silver, 2013).

2.5.2 OMA increases moisture content in soils

One potential benefit of using OMAs is to increase the capacity of the soil to retain rain and irrigation water as soil moisture (Martínez-Blanco et al., 2013). The ability for soil to hold onto water is an important factor that controls plant growth, influences plant C allocation, nutrient cycling and rate of photosynthesis (Lepsch et al., 2019; Minasny and McBratney, 2018). Some studies have shown that the application of OMAs can improve soil aggregate stability, porosity, and infiltration and water-holding capacity, and plant water status (Karami et al., 2012; Liu and Zhou, 2017). The Sand site had an increase of 28% in soil moisture content (0-0.1 m mineral soil depth) in MWC amended soils and a 37% increase from the GWC amended soils. Whereas, soil moisture increased significantly by 28% in the MWC treatment and 27% in the GWC treatment at the Loam site. The application of the OMAs in the Sand site likely increased plant available water storage by increasing water content of the soil at field capacity (Karami et al., 2012).

2.5.3 Soil inorganic N

Monthly soil sampling from 0-0.1 m showed that a fertigation event occurred around the same time as the application of OMAs, possibly contributing to the momentary spike in inorganic N in both years (Figure 4). Soil inorganic N concentrations in OMA soils increased 10-fold relative to the unamended controls after application, followed by a rapid decrease to pre-application levels after one month. Fertilization was shown to have increased inorganic N three times as much from pre-fertilization levels in the control soils at both sites.

Our results showing immediate but short-term increases in soil inorganic N pools following OMA additions is consistent with some previous OMA studies (Blackshaw et al., 2005; Eghball, 2002). Longer-term applications of compost (i.e., > 4-5 years) may be required before sustained increases in soil total inorganic N are observed (Blackshaw et al., 2005; Eghball, 2002). How long OMAs need to be applied to soil in order to achieve an elevated and sustained increase in plant available N depends on many factors such as climate, soil type, management systems, compost type, and application rates.

In a four year study focusing on annual fertilization rates (140, 224, 309 and 392 kg ha^{-1} N yr^{-1}) in mature 'Nonpareil' almond orchards in Kern County California, maximum yield was observed at the 309 kg N ha^{-1} rate (Muhammad et al., 2015). The highest application rate of 392 kg N ha^{-1} did not increase fruit, leaf or perennial tissue N suggesting that “excess” N was not used by trees. At the same time, the 309 kg N ha^{-1} rate significantly increased soil organic C and total N compared to the lower rate of 224 kg N yr^{-1} (Khalsa et al., 2020). Intensive N use in almond orchards can lead to both productivity and agricultural sustainability goals. Continuing application of OMA can provide substantial N for almond productivity that can lead to lower dependence on N fertilizer use while improving overall soil fertility and soil C storage.

2.5.4 Available N and P measured by IER

We found no significant increase in plant-available N following OMA applications using ion exchange resins (IER) placed at a 0.5-m soil depth. Nevertheless, IER did suggest higher N availability in the Loam than the Sand Site. This result is consistent with the higher soil total N concentration found in soils from the Loam site (Fig. 6b). In contrast, available P measured using this same method showed that the MWC, but not the GWC, treatment increased plant-available P after the second application. This result may be due to the very high initial P concentration in the MWC amendments used in the second year (2017; Table 1). These results suggest that certain OMA that are high in initial P may substantially increase plant available P in the plant rooting zone even when these materials are applied to the surface as in orchards. Furthermore, in contrast to the higher available N in the Loam site measured using IER, available P was substantially higher in the Sand site than the Loam site.

Our IER nutrient values were influenced by environmental and experimental factors (Binkley and Hart, 1989; Tahovská et al., 2018, 2010). The 0.5 m PVC pipe excluded plant uptake, allowing nutrients to leach to the bottom where the IER was located. Greater water transport is expected in the Sand site due to coarser soil texture versus the Loam site, facilitating nutrient transport. Differences between plant-available N and P concentrations from the IER were shown to be possibly attributed to N in OMA still in the organic state. It is uncommon for deciduous tree crops to be P deficient in California (Carlson, 1996; Warren, 1996); therefore, there is no reason for growers to apply additional P fertilizer to orchards unless to provide for the P-demands of a cover crop (Carlson, 1996).

2.5.5 Available K

The application of OMA contributed to increases in soil K availability. Previously, yard waste composts have been shown to increase soil available K because of the large proportion of woody plant material (Hartl et al., 2003; Parkinson et al., 1996). For instance, Parkinson et al. (1996) found that incorporation of 15, 30, and 50 t ha^{-1} of yard waste compost applied three times over twenty years increased soil available K by 40%, 60%, and 184% respectively. The application of manure amendments has also shown to increase available soil K. In a three-year experiment in apple orchards in Washington State, USA, researchers found that a dairy compost, at an application rate of 50 t ha^{-1}, increased available K by 185% (Forge et al., 2013). We found no differences between OMA type contributing to K availability, but both the MWC and GWC contributing to significant increases relative to the control.

Plants require large amounts of K, but the mobility of K in soils is relatively low (Carlson, 1996). Hence, one major challenge is applying sufficient amount of K to obtain crop responses in finer textured soils. Sandy soils do not have the capacity to bind K due to fewer exchange sites; consequently, leading to higher applications of fertilizer or OMA (Carlson, 1996). In a study focusing on fertilizer rates in almond orchards, Muhammad et al. (2018) concluded that almond orchard soils that had a baseline of 100-150 mg kg^{-1} of soil exchangeable K would need only an application 112 kg ha^{-1} with any fertilizer source to satisfy plant K demand. Soil exchangeable K pre-compost application in our study were 170 mg kg^{-1} at the Loam site and 100 mg kg^{-1} at the Sand site (Figure 7b.). After the experiment MWC amended soils were 240 mg kg^{-1} and 150 mg kg^{-1} in the Loam and Sand sites, respectively. The GWC amendment increased exchangeable K to 310 mg kg^{-1} in the Loam site and 150 mg kg^{-1} in the Sand site. Approximately a total of 4,304 kg K ha^{-1} were applied in 2016 and a total of 3,228 kg K ha^{-1} were applied in 2017. These calculations suggest that the application of OMA supplied sufficient amount of K for both orchards, which would allow the reduction of K fertilizer at these two sites.

2.5.6 OMA effects on CEC

We expected that the Sand site would exhibit the greatest increase in soil CEC because of the lower soil CEC in the unamended coarser textured soil (Fig. 6). In contrast to our hypothesis, we found substantial increases in soil CEC after just two years of OMA applications applied to the ground surface but only in the finer textured soil (Loam site).

As noted above, application of OMAs has been shown to increase organic C stocks, which often correlates with an increase in CEC in soils (Diacono and Montemurro, 2010). This correlation is due to the high abundance of negatively charged sites found in organic matter, especially at higher pH (García-Gil et al. 2004; Kaur et al. 2008). We also found greater increases in soil organic C in the Loam site than the Sand site, suggesting that this increase in soil CEC was driven by increases in SOM (see below).

We speculate that significant increases in soil CEC only occurred in the Loam site because the Sand site did not have enough charged surfaces (e.g., clay) to sorb the OMA-derived new soil organic matter. The higher abundance of charged surfaces in the Loam site soil protected more of the OMA-derived soil organic matter from decomposition and leaching (García-Gil et al., 2004; Kaur et al., 2008). Soils that have higher CEC have a greater capacity to store plant available nutrients, whether derived from added OMAs or from other sources (García-Gil et al., 2004). How long-lived these increases in CEC likely depend on the capacity of soil to physically protect these SOM pools from decomposition and leaching (Diacono and Montemurro, 2010). Coarser textured soils are in greater need of increases in CEC from OMA-derived organic matter because of their low clay concentrations and relatively high rates of nutrient loss from leaching (Ozores-Hampton et al., 2011). More frequent and higher application rates may be needed to provide equivalent effects of soil CEC for coarser textured soils.

2.5.7 Soil organic C and total N

Organic matter amendments that decomposed faster resulted in greater increases in surficial (0 – 0.1 m) soil C and N pools. The faster and greater loss of C and N from the GWC amendment than the MWC amendment resulted in a greater increase in soil total C and N pools, yet both OMAs increased soil C and N pools over unamended control soils. We found that soil collected from 0-0.1 m increased in soil C and N after two years of OMA application. Soil organic C and total N increased in soils at both sites. Soil C concentrations increased by an average of 70% and total N by an average of 90% in the OMA plots relative to controls. These increases in soil organic C in the OMA plot may play an essential role in the nutrient content and availability (Li et al. 2017; Liu and Zhou 2017; Zhao et al. 2009).

We found in our study the impact of OMA additions contributed to increases in organic C and total N in only 2 years with typical agronomic OMA application rates. Longer and higher rates of OMA application may continue to contribute to organic C and N pools. In a fourteen-year nectarine orchard study conducted in Italy, Baldi et al. (2018) found that two application rates (5 Mg dry weight of compost (DW) ha^{-1} yr^{-1} and 10 Mg DW ha^{-1} yr^{-1}) increases organic C concentrations in the top 0.65 m of soil. The highest application rate contributed to 60% of C sequestration from the amendment source over fourteen years. Some studies, such as in Glover et al. (2000), reported no changes in SOC stocks in the top 0.15 m after 4 years of application of poultry manure and bark mulch in apple orchards. It was concluded that the high initial C levels and historical land use as a dairy farm decreased decomposition rates, resulting in these OMAs contributing little to SOC pools (Powlson and Jenkinson 1981). In contrast, Mbau et al. (2015) found an increase soil organic C and total N in the top 0.2 m of soil after 17 months from the application of six different composts at a rate of 5 Mg ha^{-1} in nutrient deficient maize field soils. The differences among these studies are most likely due to the chemical composition of the OMA used in the study, the rate of application, the length of the study, or sampling depth.

2.6. Conclusion

Our results have important implications for managing agricultural soils, particularly permanent crops in California's Central Valley. Our study demonstrates that composts derived from readily available dairy cow manure and green yard waste can significantly improve soil fertility after one or two annual applications, and that fertility gains appear to be more dependent on soil texture than the nutrient concentrations of the OMA. By improving soil fertility, OMAs have the potential to provide co-benefits to growers, such as sustaining the productivity of their soils, reducing inorganic fertilizer usage, recycling on site, and increasing yields.

Soil fertility is the ability of soil to sustain agricultural plant growth, resulting in sustained and consistent high-quality yields. The continuation of this study to tree maturity would allow the evaluation of the impact of these OMAs on crop yields and quality. Although we found soil nutrient and water content differences resulting from OMAs at two sites with differing textures, we recognize that this study did not evaluate other physical and biological components of soil fertility. Multiyear field studies are needed to explore long-term benefits of OMAs in orchard systems with different environmental variables in order to fully assess OMA influences on long-term soil fertility and crop yield.

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2.8 Tables

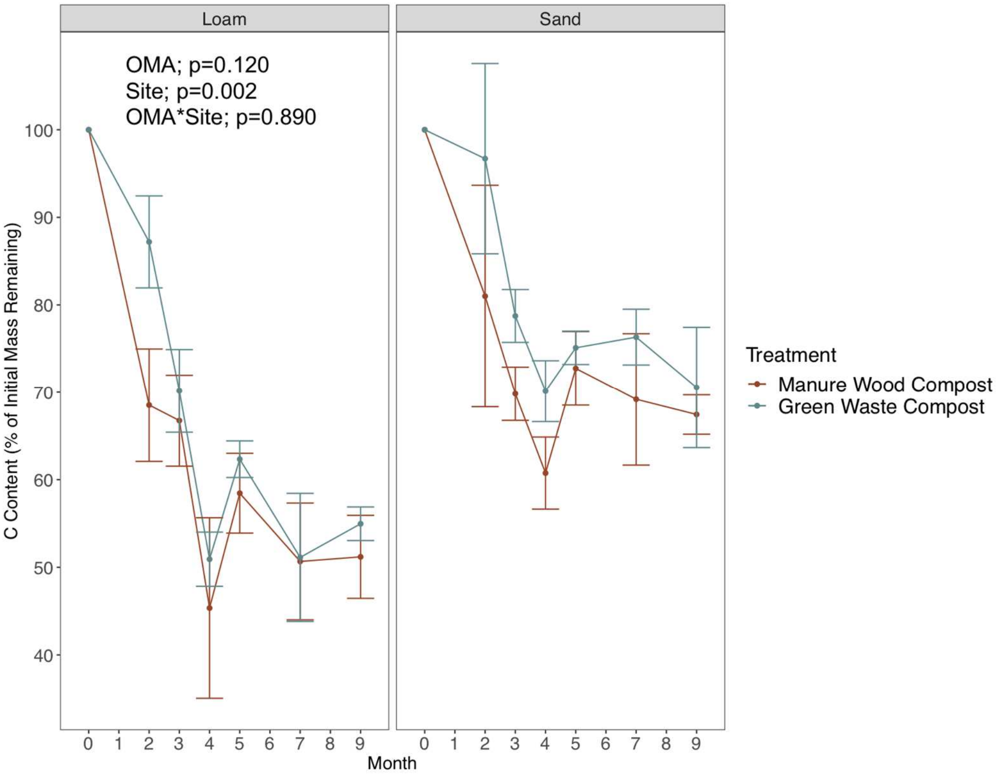
Table 2-1. Mean (± standard error, n = 3 - 4) total nutrient concentrations in the two organic matter amendments (OMA) applied to almond orchards of contrasting soil texture during 2016 and 2017. For each mean nutrient concentration or nutrient ratio, values followed by different lower case letters denote significant differences (one-factor ANOVA, Tukey mean comparison test; $p \leq 0.05$) among the OMAs applied that year. MWC = manure wood chip compost, GWC = green waste compost.

Year/ OMA	**C ($g\ kg^{-1}$)**	**N ($g\ kg^{-1}$)**	**C/N ($kg\ kg^{-1}$)**	**P ($g\ kg^{-1}$)**	**K ($g\ kg^{-1}$)**	**S ($g\ kg^{-1}$)**	**Ca ($g\ kg^{-1}$)**	**Mg ($g\ kg^{-1}$)**	**Fe ($g\ kg^{-1}$)**	**B ($mg\ kg^{-1}$)**	**Cu ($mg\ kg^{-1}$)**	**Mn ($mg\ kg^{-1}$)**	**Zn ($mg\ kg^{-1}$)**
2016													
MWC	$246^{b} \pm 4$	$13^{a} \pm 0$	$20^{b} \pm 0$	$4^{a} \pm 0$	$16^{c} \pm 0$	$3^{a} \pm 0$	$26^{a} \pm 1$	$6^{b} \pm 0$	$16^{b} \pm 0$	$54^{c} \pm 1$	$78^{a} \pm 2$	$305^{a} \pm 7$	$184^{a} \pm 10$
GWC	$303^{c} \pm 9$	$23^{b} \pm 1$	$13^{a} \pm 0$	$5^{b} \pm 0$	$12^{b} \pm 0$	$4^{c} \pm 0$	$34^{b} \pm 1$	$6^{a} \pm 0$	$12^{a} \pm 0$	$42^{b} \pm 1$	$132^{c} \pm 4$	$384^{bc} \pm 31$	$332^{c} \pm 6$
2017													
MWC	$153^{a} \pm 4$	$13^{a} \pm 0$	$12^{a} \pm 0$	$10^{c} \pm 0.1$	$16^{c} \pm 0$	$4^{c} \pm 0$	$32^{b} \pm 0$	$7^{c} \pm 0$	$16^{b} \pm 0$	$56^{c} \pm 1$	$120^{bc} \pm 1$	$440^{c} \pm 5$	$237^{b} \pm 1$
GWC	$295^{c} \pm 6$	$24^{b} \pm 1$	$12^{a} \pm 0$	$5^{b} \pm 0.2$	$11^{a} \pm 0$	$4^{b} \pm 0$	$33^{b} \pm 1$	$5^{a} \pm 0$	$12^{a} \pm 0$	$38^{a} \pm 1$	$115^{b} \pm 5$	$333^{ab} \pm 9$	$324^{c} \pm 9$

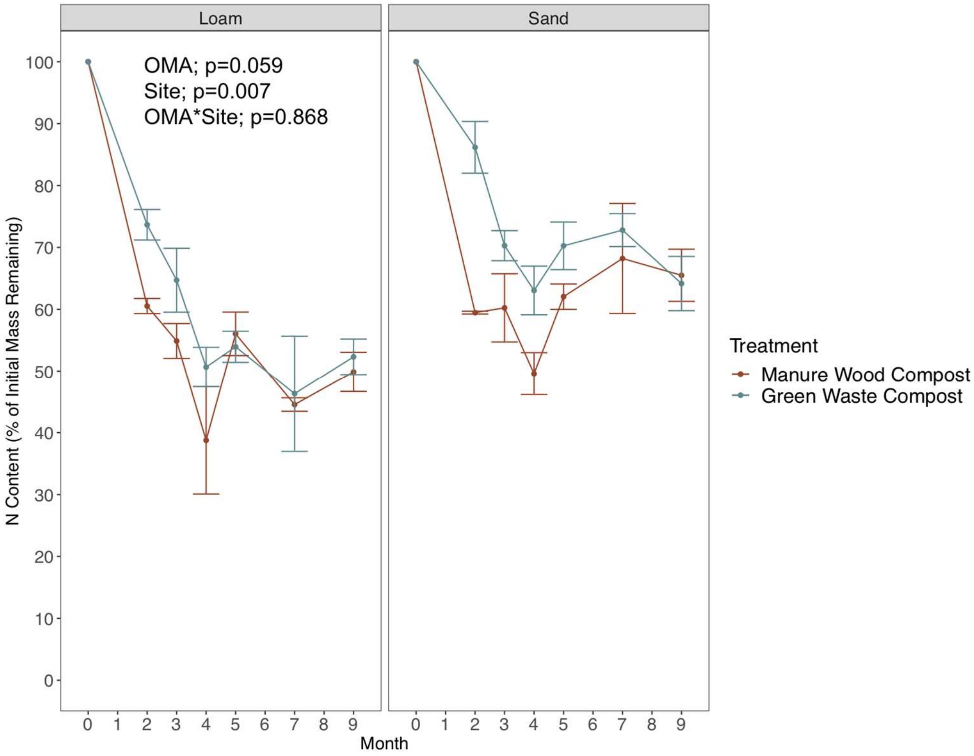
2.9. Figures

Figure 2-1. Effect of different OMAs application and soil texture on soil (0-0.1 m) (**a**) organic carbon (C), (**b**) total nitrogen (N), (**c**) total phosphorus (P), and (**d**) total potassium (K) remaining in Organic Matter Amendments (OMAs) during decomposition in two almond orchards. Statistics describe a two-way repeated measures ANOVA ($p<0.05$). Error bars denote ± one standard error of the mean.

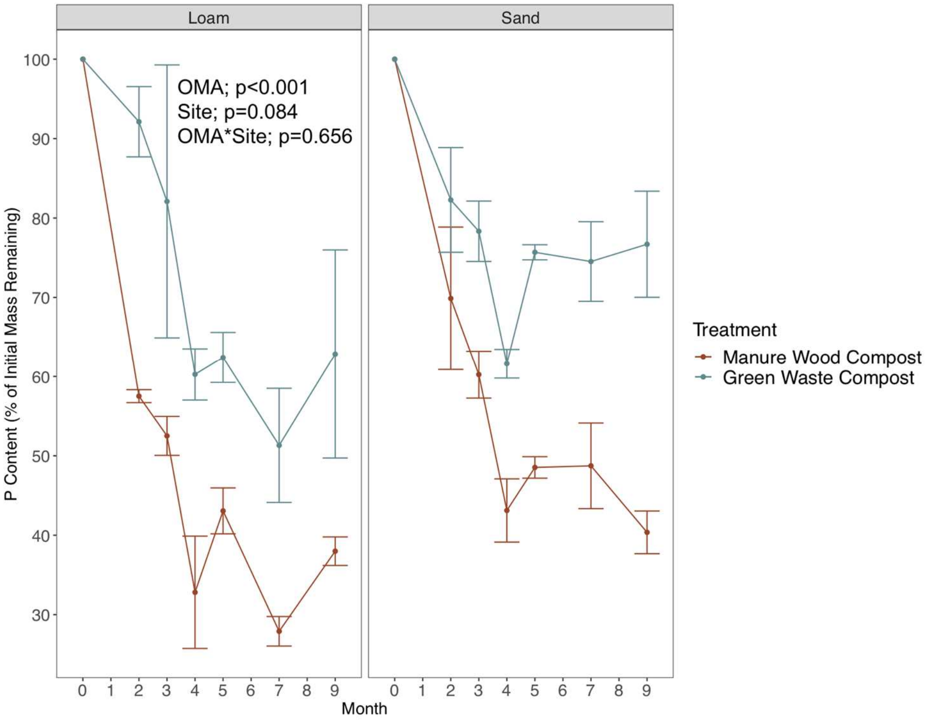
a)



b)


Loam
Sand
OMA; p=0.059
Site; p=0.007
OMA*Site; p=0.868
N Content (% of Initial Mass Remaining)
100
90
80
70
60
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9
Month
Treatment
Manure Wood Compost
Green Waste Compost

c)


Loam
Sand
OMA; p<0.001
Site; p=0.084
OMA*Site; p=0.656
P Content (% of Initial Mass Remaining)
100
90
80
70
60
50
40
30
0 1 2 3 4 5 6 7 8 9
Month
Treatment
Manure Wood Compost
Green Waste Compost

d)

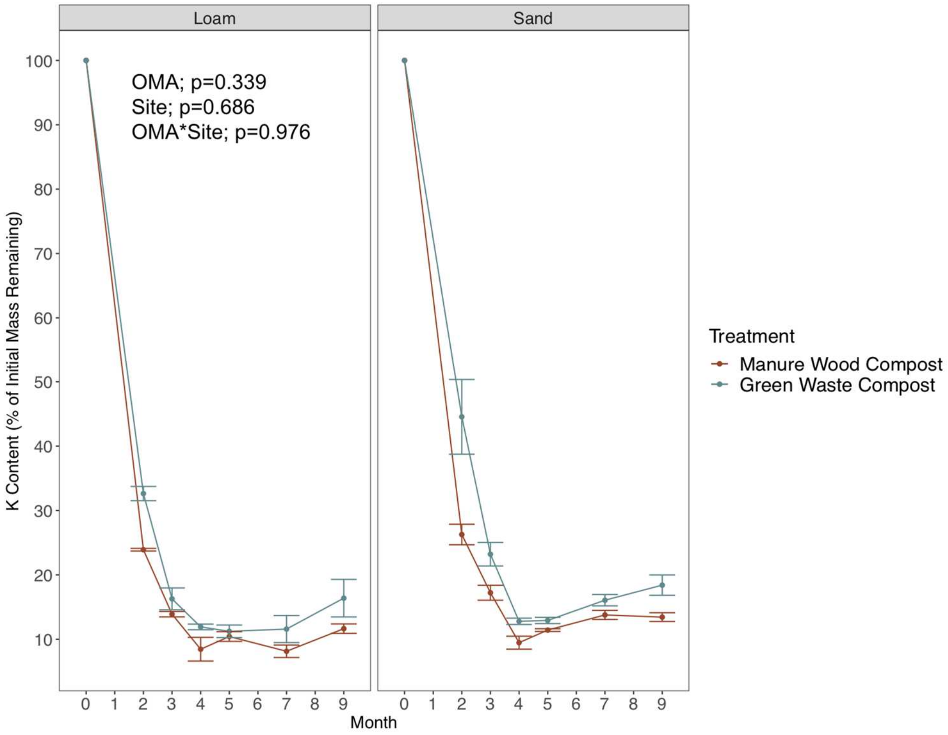

Loam
Sand
OMA; p=0.339
Site; p=0.686
OMA*Site; p=0.976
K Content (% of Initial Mass Remaining)
100
90
80
70
60
50
40
30
20
10
0 1 2 3 4 5 6 7 8 9
0 1 2 3 4 5 6 7 8 9
Month
Treatment
Manure Wood Compost
Green Waste Compost

Fig 2-2. Soil moisture (0-0.1 m) during the two-year study period. Symbols denote means and error bars are standard errors of the mean (n = 3 - 4). A two-way repeated measures ANOVA was performed ($p<0.05$).

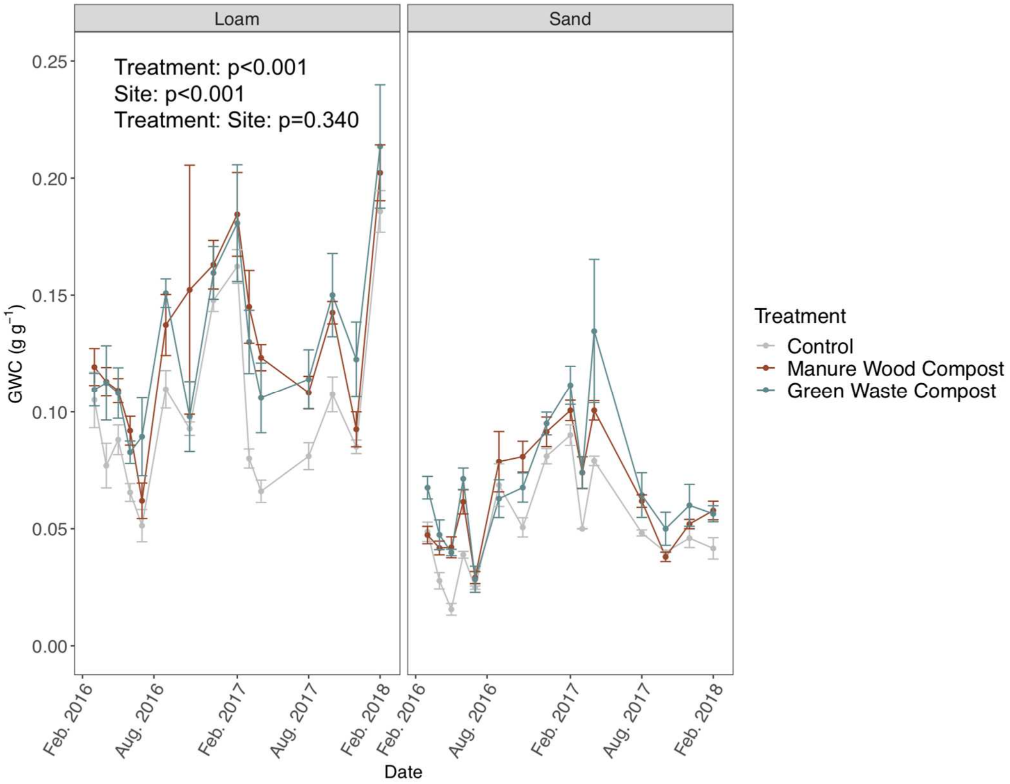


Fig 2-3. Soil (0-0.1 m) inorganic nitrogen (N) dynamics during the two-year study period. Symbols denote means and error bars are standard errors of the mean (n = 3 - 4). The red arrows denote the timing of the application of the OMAs and the black arrows denote the timing of fertilization events (see Materials and Methods). A two-way repeated measures ANOVA was performed (p<0.05). Error bars denote ± one standard error of the mean.

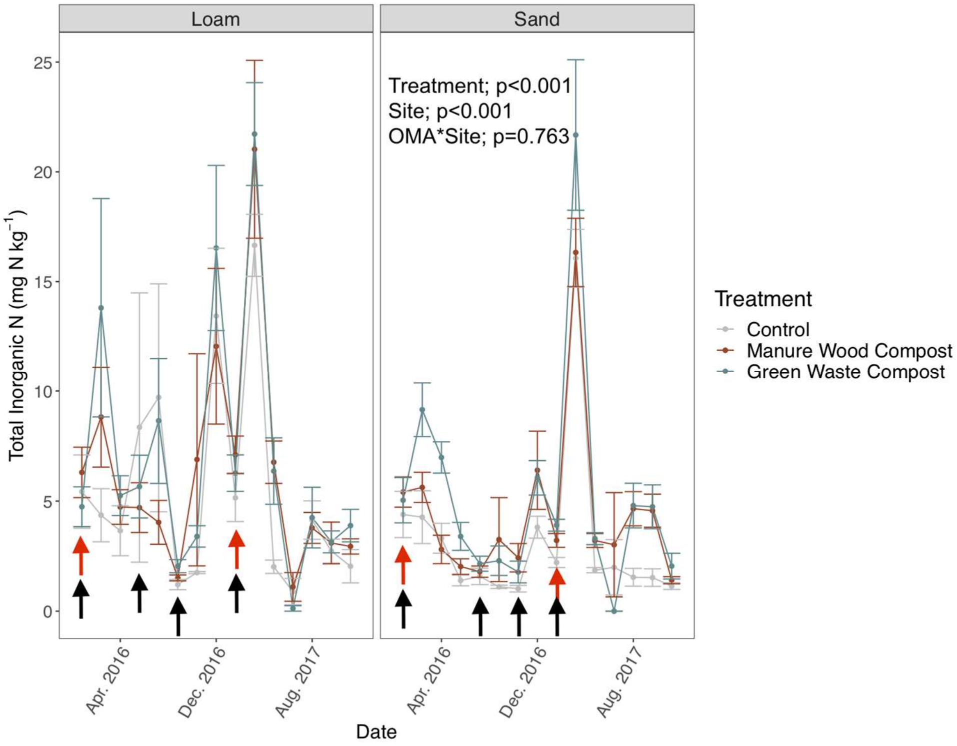
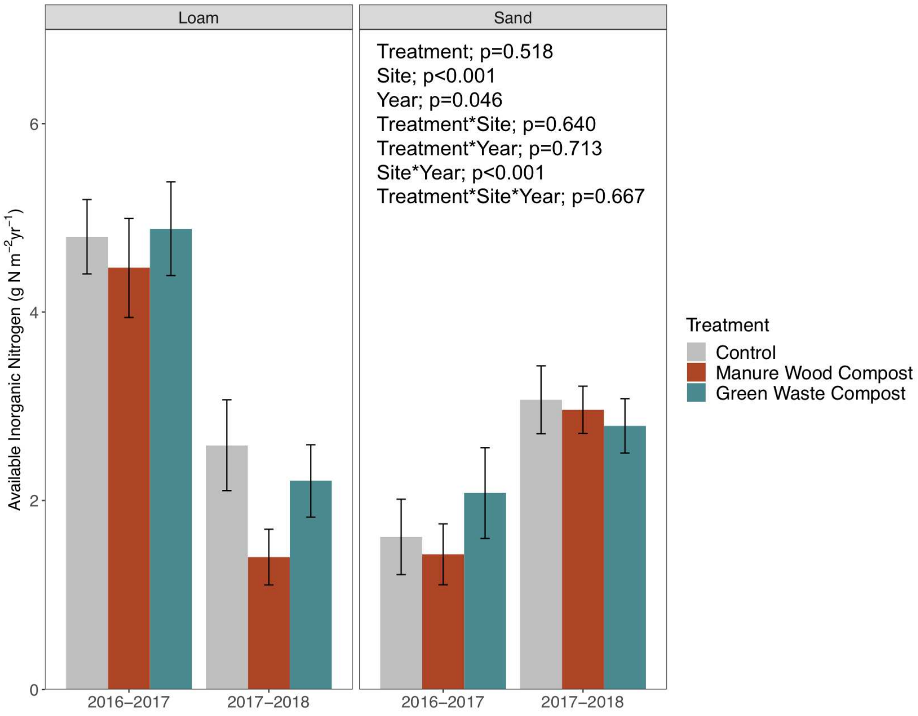


Fig 2-4. Mean inorganic nitrogen (N) and phosphorus (P) fluxes (over a two-year period) determined by adsorption on ion exchange resin bags placed at a 0.5-m mineral soil depth in the absence of plant nutrient uptake. A three-way repeated measures ANOVA was used ($p<0.05$). Colored bars denote mean values and error bars denote ± one standard error of the mean ($n = 8$).

a)



b)

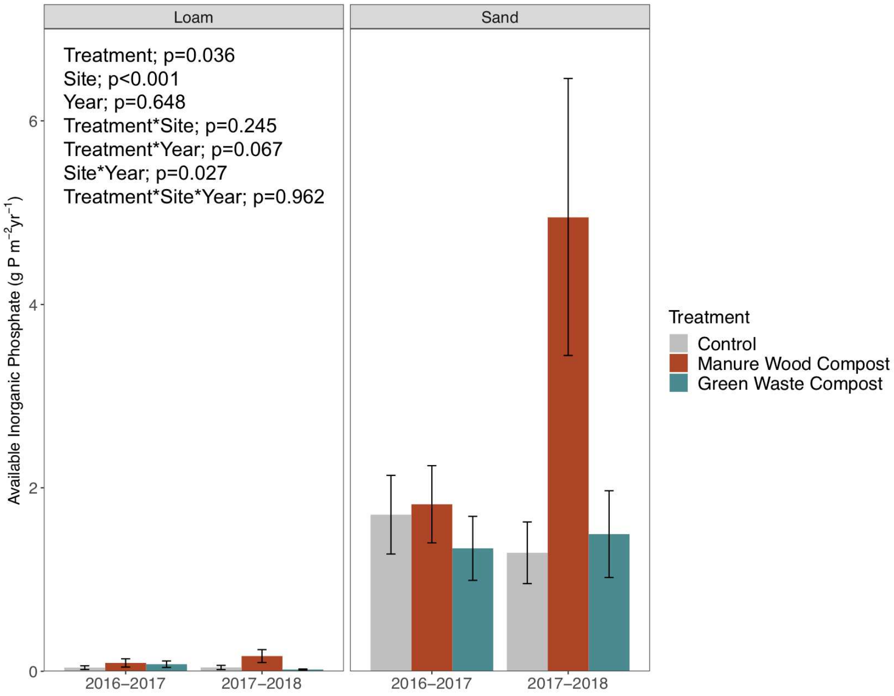
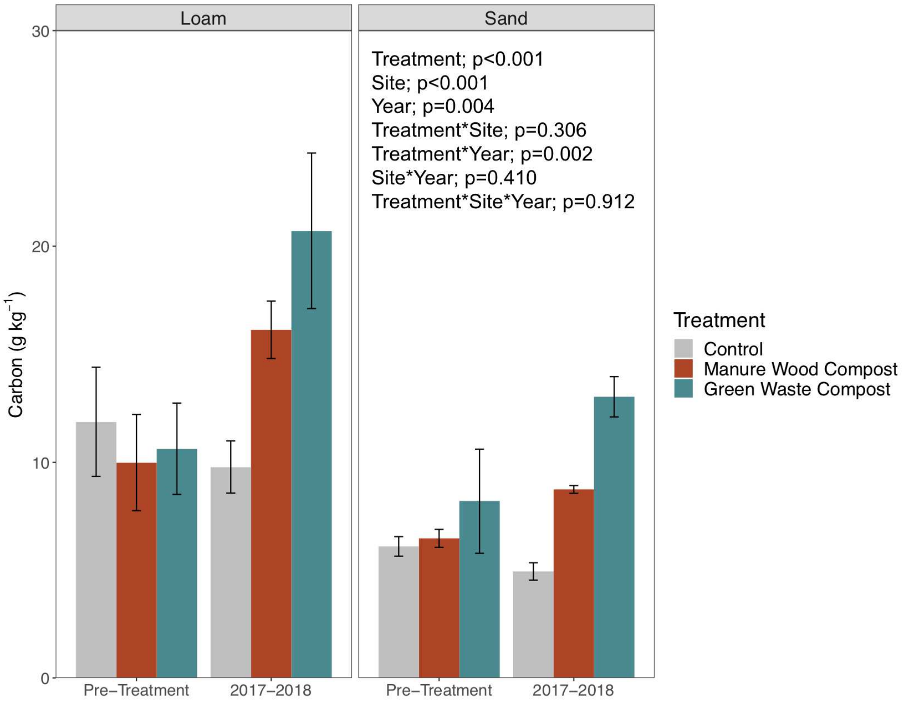

Loam
Sand
Treatment; p=0.036
Site; p<0.001
Year; p=0.648
Treatment*Site; p=0.245
Treatment*Year; p=0.067
Site*Year; p=0.027
Treatment*Site*Year; p=0.962
Available Inorganic Phosphate (g P m⁻²yr⁻¹)
0
2
4
6
2016–2017
2017–2018
2016–2017
2017–2018
Treatment
Control
Manure Wood Compost
Green Waste Compost

Fig 2-5. Soil (0-0.1 m) organic carbon (**a**) and total nitrogen (**b**) before (2016) and after (2018) two years of application of two different organic matter amendments to almond orchards of contrasting soil texture. A three-way repeated measures ANOVA was used ($p<0.05$). Colored bars denote mean values and error bars denote ± one standard error of the mean (n = 3 - 4).

a)



b)

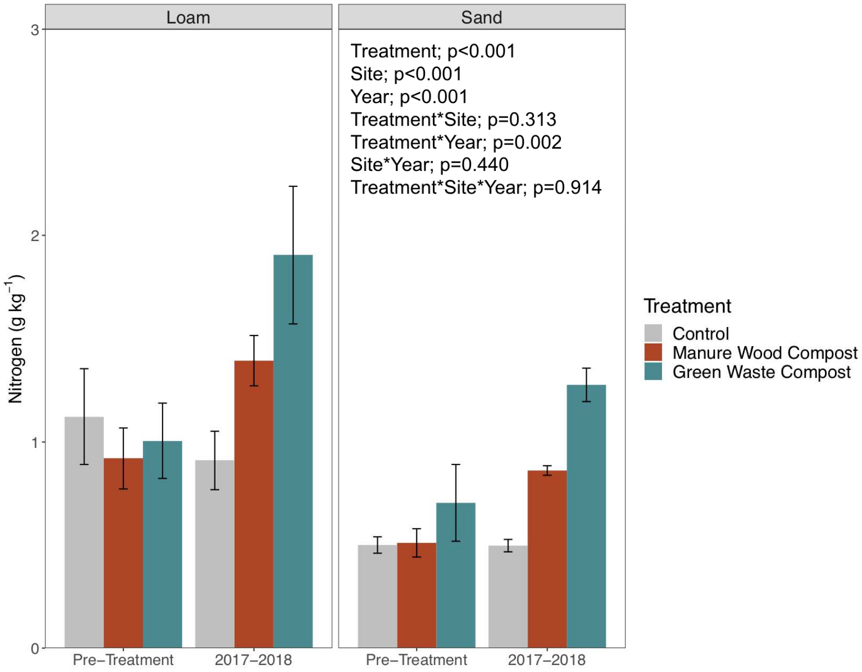
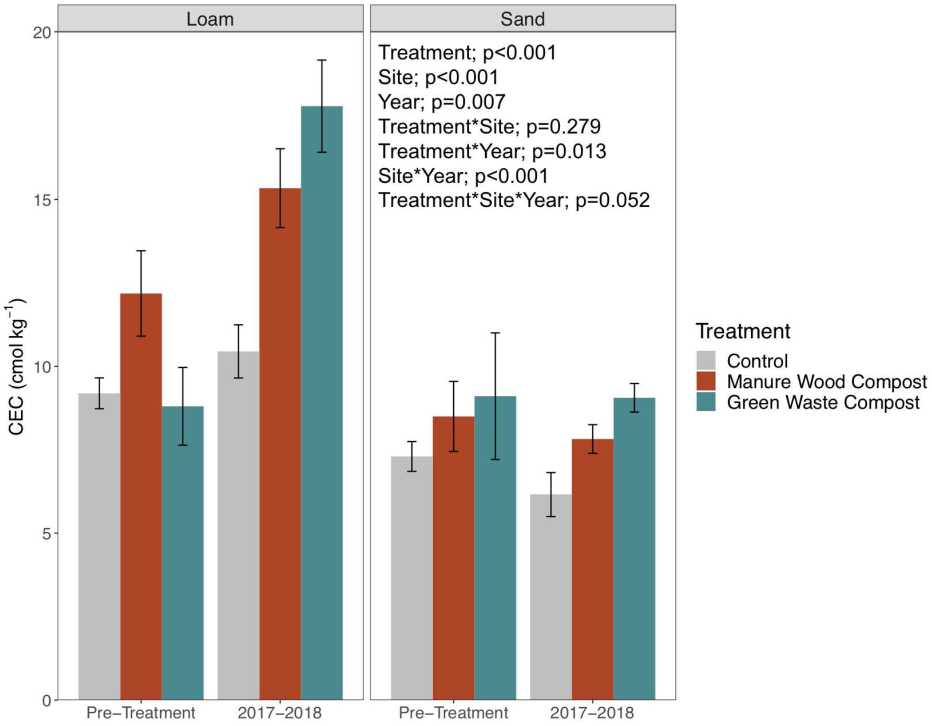
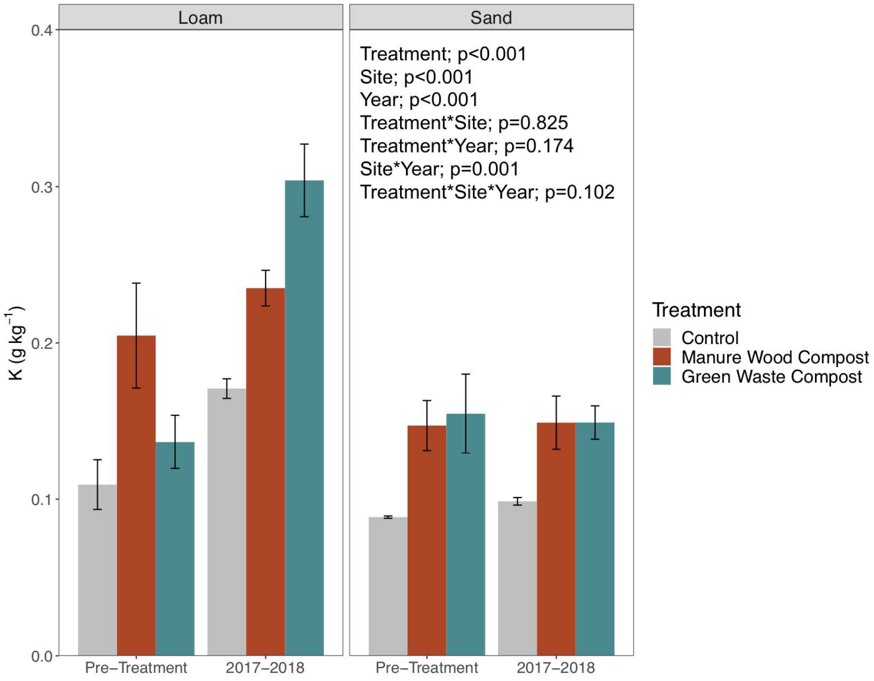

Loam
Sand
Treatment; p<0.001
Site; p<0.001
Year; p<0.001
Treatment*Site; p=0.313
Treatment*Year; p=0.002
Site*Year; p=0.440
Treatment*Site*Year; p=0.914
Nitrogen (g kg-1)
0
1
2
3
Pre−Treatment
2017−2018
Pre−Treatment
2017−2018
Treatment
Control
Manure Wood Compost
Green Waste Compost

Fig 2-6. **a**) Changes in soil (0-0.1 mm) cation exchange capacity (CEC) and **b**) potassium (K) before (2016) and after (2018) two years of application of two different organic matter amendments to almond orchards of contrasting soil texture. A three-way repeated measures ANOVA was used ($p<0.05$). Colored bars denote mean values and error bars denote ± one standard error of the mean (n = 3 - 4).

a)



b)


Loam
Sand
Treatment; p<0.001
Site; p<0.001
Year; p<0.001
Treatment*Site; p=0.825
Treatment*Year; p=0.174
Site*Year; p=0.001
Treatment*Site*Year; p=0.102
0.4
0.3
0.2
0.1
0.0
K (g kg-1)
Pre−Treatment
2017−2018
Pre−Treatment
2017−2018
Treatment
Control
Manure Wood Compost
Green Waste Compost

3 Soil carbon response to long-term biosolids application

3.1 Abstract

A study was conducted in three agroecosystems in California (Sacramento, Solano, and Merced counties) that received biosolids applications for twenty-years. Management varied in application rates and frequencies, resulting in average cumulative amount of biosolids applied of 74 (Solano), 105 (Merced), and 359 (Sacramento) Mg biosolids$_{\text{dry}}$ /ha., resulting in the addition of 26 (Solano), 36 (Merced), and 125 (Sacramento) Mg biosolids-C/ha. Measurements included SOC and total nitrogen (N) concentrations from 0-100 cm, and microbial biomass C (MBC) and N (MBN) from 0-30 cm in biosolids-amended and controls. Biosolids treatments had greater amounts of soil organic carbon (SOC) and total N at all sites, and microbial biomass C and N at Sacramento and Solano. Largest increases in SOC were at the site that received the lowest cumulative loading rate of biosolids (Solano), where SOC content to 100 cm was 50% greater in amended soils ($p<0.001$). Net changes in soil C stocks to 30 cm were 0.4±0.1 (Solano), -0.04 ± 0.1 (Merced), and 0.3±0.2 (Sacramento) Mg C ha^{-1} yr^{-1}. These values change when considering deeper soil depths (0-100 cm) to 0.5±0.1 (Solano), 0.2 ±0.2(Merced), and 0.216 ±0.2 (Sacramento) Mg C ha^{-1} yr^{-1}, reflecting differences in C stocks changes in surface and subsurface soils across sites. Rates of C storage per dry Mg of biosolids per year applied were 1±0.2 (Solano), 0.5±0.4 (Merced), and 0.04±0.1 (Sacramento). Our results suggests that local controls on soil C stabilization are more important than amendment application amount at predicting climate benefits, and that accounting for soil C changes below 30 cm can provide insight for sequestering C in agroecosystems.

Keywords: carbon sequestration, biosolids, soil carbon, equivalent soil mass

3.2. Introduction

Biosolids application is an effective way to increase soil fertility and reduce fertilizer use by recycling nutrients onto agricultural lands (Avery et al., 2018). Biosolids contain plant essential nutrients such as phosphorus, plant-available N, and micronutrients (Cogger et al., 2013; Fernandes et al., 2005; Nicholson et al., 2018). When applied, biosolids improve soil physical properties (Kominko et al., 2017; Ramulu, 2001; Veeresh et al., 2003). Biosolids also promote biological soil processes such as nutrient mineralization and soil respiration (Carbonell et al., 2009; García-Gil et al., 2004; Lloret et al., 2016).

Land application of biosolids has also been shown to promote soil C sequestration in some cases (Spargo et al., 2008; Tian et al., 2009). Increased SOC in surface soils, typically to 5 or 30 cm depth, has been assessed with long-term, repeated application of biosolids in wheat production systems (Brown et al., 2011; Pan et al., 2017; Pitombo et al., 2015; Tian et al., 2013, 2009). Some studies report sustained increases in soil C and plant production after a one-time application of biosolids (Brown et al., 2011; Wijesekara et al., 2017), while others detect carryover in plant responses but no lasting change in soil C (Avery et al., 2018). Across existing studies, the magnitude of C change in soils amended with biosolids is variable due to climatic conditions, management, and properties of the soil and biosolids (Wijesekara et al., 2017). Therefore, it is imperative for more localized, comparative and mechanistic field research in order to assess the potential to use biosolids as a means to increase soil C.

While the benefits of biosolids application are evident in many agricultural soils, most studies sample only the top 30 cm or less of soil (Pan et al., 2017; Pitombo et al., 2015; Stewart et al., 2012; Tian et al., 2013, 2009). There have been relatively few soil C studies that have examined deep soil C, regardless of the management practice examined. When deep (>30 cm) soil C is included, it furthers our understanding of organic matter dynamics and C accounting of management practices (Poeplau and Don, 2015; Tautges et al., 2019; Yost and Hartemink, 2020). Shallow sampling leads to an incomplete understanding of the effect of biosolids land application on soil C storage and can result in a under-estimation of a soil's potential to sequester C (Lorenz and Lal, 2005). As SOC moves into subsoils, the turnover rate tends to decrease (Tautges et al., 2019). Over half of SOC in a profile can be stored below 30 cm (Harrison et al., 2011). Additionally, subsoils contain higher concentrations of clays and organo-mineral complexes that can contribute to soil C stabilization over long timescales ranging anywhere from years to hundreds of years depending on soil conditions (Kögel-Knabner et al., 2008).

It is important to determine under which agricultural contexts biosolids increase C in order to fully understand the highest and best use of this underutilized organic resource. The purpose of this study was to determine the effects of long-term (20 y) application of biosolids on soil C stocks to 100 cm depth in nine sites situated within three agricultural ecosystems in California. We hypothesized that biosolids increase SOC content in shallow depths due to incorporation of biosolids-C and enhanced plant production. In addition, biosolids promote biological soil health as indicated by an increase in soil microbial biomass (Charlton et al., 2016; Fernandes et al., 2005). We also hypothesize that SOC will increase below the depth of biosolids incorporation (~30 cm).

3.3. Materials and Methods

3.3.1. Study Sites and Experimental Design

The study sites were situated in Northern California, including locations in Sacramento County, CA (38°20'06.3"N, 121°10'06.5"W), Solano County, CA (38°11'52.3"N, 121°45'38.0W), and Merced County, CA (37°04'20.3"N, 120°31'43.0"W). Sampling was replicated at three fields within each site and included unamended control and amended transects in each field (n=9). At each study site, three paired transects were established consisting of biosolids amended and unamended control soils. Transects were selected using information from records of biosolids application to the site, USDA-NRCS soil maps, and discussions with the land manager at each site. This information helped identify a control with comparable soil properties and management history within the same field as biosolids-amended soils. Each paired transect had the same soil series, grazing or cropping practices, water management, and prior land management histories. Neither biosolids nor unamended control transects received inorganic fertilizers. Samples were collected using a 57 mm-diameter auger at five depth increments, 0-10 cm, 10-30 cm, 30-50 cm, 50-75 cm, and 75-100 cm, at every ten meters along a 100-m transect. Transect starting points and bearings were randomly selected from within each field, excluding a 5 m buffer from edges. A total of 900 soil samples were collected and transported to UC Merced for laboratory analysis.

The Sacramento site consisted of flood-irrigated annual grasslands managed for grazing beef cattle. Livestock was rotated every 60-70 days depending on feed availability. Soils are classified as Alfisols from the Hicksville and Corning series. The Solano site consists of rainfed annual grasslands used for grazing lambs and beef cattle. Grazing intensity is 0.3 animal units ha^{-1}. Livestock are rotated seasonally and based on feed availability. Soils are classified as Alfisols from the Antioch and Pescadero series and Vertisols from the Altamont soil series. The Merced site is flood irrigated and managed for livestock feed crops consisting of 1 y corn and 4 y alfalfa rotations for livestock silage and were tilled after each rotation. Soils are classified as Mollisols from the Pozo series and Alfisols from the Fresno series. Soil texture, pH, MAP, MAT, and biosolids application rates at each site are shown in Supplemental Table 1.

Biosolids were applied at each site according to county, state, and federal regulations for biosolids land application. Class B, dewatered biosolids were applied based on local agronomic rates determined by plant N demands, soil N stocks, and potentially available N concentrations of biosolids. Each site received biosolids from multiple wastewater treatment plants over the 20-year timeframe (Sacramento: yearly 1999-2018; Solano: every three years 2005-2017; Merced: every five years 1995-2011). Biosolids were surface applied and incorporated via tillage to a soil depth of approximately 30 cm. Controls in the Sacramento and Solano sites that did not receive biosolids application were not tilled; however, Merced was tilled since alfalfa and corn were still grown on the control soil. Application of biosolids is prohibited within a pre-determined distance from certain areas, including property lines, roadways, and water supply wells, based on county-level regulations. We selected sites that included a comparative unamended area (hereafter referred to as "control") immediately adjacent to the area where biosolids were applied.

3.3.2. Biosolids characteristics

We used a combination of land application records and analyses of new samples to characterize biosolids and to estimate the contribution of biosolids to changes in soil C and N. Land application records included the amount of biosolids applied annually within each field where transects were placed, as well as nutrient concentrations of the biosolids. We calculated the total amount of biosolids dry mass applied over the 20-year period and summarized application frequency for each transect (Table 1). We determined the amount of biosolids-N applied to each amended transect by multiplying biosolids dry mass by total nitrogen. Records did not include biosolid-C content; hence, fresh, dewatered biosolids samples were analyzed in order to estimate the amount of C applied.

Samples of Class B biosolids were collected from ten wastewater treatment plants that provide biosolids to farmers in the study region (n=10). These wastewater treatment plants generate biosolids using either mesophilic or thermophilic anaerobic digestion technology. The core infrastructure and technologies used to produce biosolids at wastewater treatment plants in this region has not changed significantly over the course of this study (R. Batjiaka, *pers. comm.)*. Average dry weather flows ranged from 5.7 to 182 million L d^{-1}. Treatment plants served areas with populations ranging from 37,000 to 635,000 people (1995-2011; R. Batjiaka, *pers. comm.)*. Biosolids samples were frozen at -20°C prior to C, N, and nutrient analysis at the UC Davis Analytical Laboratory. Subsamples of the biosolids were freeze-dried, finely ground, and analyzed for C and N on an elemental analyzer (TruSpec CN Analyzer, LECO, Michigan, USA). We estimated the amount of biosolids-C applied to each amended transect by multiplying cumulative biosolids dry mass applied by mean biosolids C concentration. Additional subsamples were digested using nitric acid/hydrogen peroxide microwave digestion and then analyzed on an Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) for total phosphorus (P), potassium (K), sodium (Na), sulfur (S), calcium (Ca), magnesium (Mg), iron (Fe), boron (B), copper (Cu), manganese (Mn), and zinc (Zn; Meyer and Keliher, 1992; Sah and Miller, 1992).

3.3.3. Soil analyses

Soil pH was determined for all 900 samples and was measured in a 1:2 soil to deionized water ratio (McLean, 1982). Soils from the control transects were used to determine soil texture using the hydrometer method (Gee and Bauder 1986), as organic matter amendments do not change soil texture (Ryals et al. 2014). Field replicates were composited by depth for each transect and run in duplicate for soil texture at UC Merced (n=15 per site).

We analyzed soils for total C, organic C, and total N from each depth increment (0-10, 10-30, 30-50, 50-75, and 75-100 cm) from the nine paired transects. Soil samples were air dried and sieved at 2 mm to remove coarse fragments and roots (Kuzyakov et al., 2001). Gravimetric water content of air-dried soil was determined by drying a 10 g of soil at 105 ºC until mass was stable. Masses of soil samples were corrected for moisture using the gravimetric water content; then ground finely using a mortar and pestle. Soil total C and N concentrations were measured on a Costech ECS 4010 CHNS-O Elemental Analyzer (Valencia, CA, USA; NIST standards included: peach leaves, USGS 40, USGS 41a, and costech acetanilide) coupled to a Thermo Scientific Delta-V Plus continuous flow isotope ratio mass spectrometer (Waltham, MA, USA) at the UC Merced Stable Isotope Laboratory. Soils from the Merced site were further treated using the acid fumigation method due to effervescence upon addition of 4 N HCl, indicating the presence of soil inorganic carbon (Harris et al., 2001). Acid-treated soils were analyzed on the Costech ECS 4010 CHNS-O Elemental Analyzer (Valencia, CA, USA; NIST standards included: peach leaves, USGS 40, USGS 41a, and costech acetanilide) coupled to a Thermo Scientific Delta-V Plus continuous flow isotope ratio mass spectrometer (Waltham, MA, USA) at the UC Merced Stable Isotope Laboratory to determine the concentration of SOC. Soil inorganic C (SIC) was calculated by subtracting the acid-treated C concentrations from total soil C concentrations.

Long-term application of biosolids may alter soil bulk density over time (Albaladejo et al., 2008). Therefore, it is imperative to account for changes in bulk density caused in order to get an accurate measurement for C and N stock; however, using a "fixed-depth" method may lead to errors when bulk density differs between treatments and can be time consuming when sampling across many fields and sites (Wendt and Hauser, 2013). In order to obtain a valid comparison of SOC and total N stocks, we used the equivalent soil mass method (Ellert and Bettany, 1995; Wendt and Hauser, 2013). Briefly, soil mass was calculated by dividing the dry soil sample mass of the depth layer by the area of the sampled by the auger. Then the mass of the soil was multiplied by the SOC concentration of that soil sample depth layer. The data was then fitted with a cubic spline to determine SOC and total N mass per area.

3.3.4. Microbial Biomass

The chloroform fumigation method is an effective measure of how much C and N is associated to microbial biomass. We analyzed three out of ten replicates per transect from the 0-10 cm and 10-30 cm depth intervals, where the majority of microbial activity occurs, for microbial biomass C (MBC) and N (MBN; (Fernandes et al., 2005). Soils were extracted within 24 hours of sampling using the chloroform fumigation method (Vance et al. 1989). Extractions were stored at -20°C until analyzed for dissolved organic C and N on the Total Organic Carbon Analyzer (Shimadzu TOC-Vcsh, Kyoto, Japan; TOC) with a total N module at the UC Merced Environmental Analytical Laboratory. Soils from the Merced site had high levels of inorganic C, and the extractions were treated with HCl for analysis. Microbial biomass C was calculated as the difference between C content of non-fumigated and fumigated soils. The same approach was used to calculate MBN. We used an extraction efficiency factor of (k_{eC}) of 0.45 for MBC and MBN (k_{eN} ; Beck et al., 1997).

3.3.5. Rates of soil organic C sequestration

Rates of SOC sequestration were calculated for each paired transect at each site (n =9) using three approaches. First, we determined annual rates of SOC sequestration on a mass per area basis (e.g., Mg C ha^{-1} y^{-1}) to a 30 cm depth. Differences in cumulative SOC from 0-30 cm between amended and control soils were calculated, and divided by 20 y. Second, the same mass per area of field site per year approach was used for the cumulative 0-100 cm soil depth. Comparing results from different approaches allowed us to compare the relative importance of soil depth in accounting for management-induced SOC sequestration. Third, we determined rates of SOC sequestration based as a function of the total amount of biosolids applied. For each transect, average treatment difference in SOC were divided by total amount of biosolids applied, resulting in a sequestration value in units of mass of C per mass of biosolids (e.g., Mg C/ Mg biosolids).

3.3.6. Statistical analyses

Statistical tests were performed using RStudio 3.3.1. A Shapiro-Wilk test was performed to determine the normality, data that did not meet the assumptions of normality were $\log_{10}$ transformed. A one-way analysis of variance (ANOVA) was used to determine statistically significant treatment effects on elemental composition of biosolids samples. Analyses included transect as blocking effect and were determined separately for each site and depth. Data for pH, MBC and MBN, inorganic C, organic C and total N content were not normally distributed after $\log_{10}$ transformation; hence, non-parametric statistical analyses, the Kruskal-Wallis test, was performed. Multiplicative and additive error propagation was determined for statistical analyses. Data that were more than two standard deviations away from the mean were classified as outliers (Stephenson, 2003). Statistical significance was established a prior at $\alpha = 0.05$, marginal significance was determined to be from $\alpha > 0.05$ to $\alpha < 0.10$. Data are reported as mean values followed by ±SE.

Cumulative soil C content from 0-100 cm was determined by adding the C content of each of the five depth increments. To determine treatment differences in the top 30 cm, SOC content of the 0-10 cm and the 10-30 cm depth increments were added. Rate of C storage was determined by subtracting biosolids amended C content from the control C content (determined from 0-30 cm and 0-100 cm), and dividing by amount of biosolids applied. Average rates of C storage were then averaged between transects within each of the three sites in order to determine site differences. A one-way ANOVA was performed to determine how rates of C storage differed between sites. Microbial biomass concentrations were converted to content at each depth increment by multiplying soil mass. Microbial biomass C content was then added from 0-10 cm and 10-30 cm. Microbial biomass to SOC ratios were calculated then multiplied by 100 to get MBC:SOC percentages as described in Sparling (1992).

3-4. Results

3.4.1. Biosolids characterization

Despite the wide variation in daily flow rates and size of populations served by the ten wastewater treatment plants, elemental composition of fresh biosolids samples was consistent. Nutrient composition of fresh biosolids samples was similar except for elemental S (p=0.04; Supplementary Table 2). Carbon concentrations of fresh biosolids ranged from 338-443 mg/kg, and total N concentration of biosolids ranged from 51-75 mg/kg. Biosolid C:N ratios ranged from 6 to 7.

An estimated total of 125±12 Mg biosolids-C/ha (359±35 Mg $\text{biosolids}_{\text{dry}}$/ha) was added in the Sacramento site over 20 years. At this site, biosolids were applied frequently, on an annual basis, and at relatively high rates. Biosolids were applied at lower rates and less often; every three or five years at Solano and Merced, respectively. The total amount of C applied from biosolids over 20 years was 26±4 Mg biosolids-C/ha (74±10 Mg $\text{biosolids}_{\text{dry}}$/ha) in Solano and 36±9 Mg biosolids-C/ha (105±27 Mg $\text{biosolids}_{\text{dry}}$/ha) in Merced (Table 1). During the 20-y period, 19±2 Mg biosolid-N/ha was applied at Sacramento, 4±1 Mg biosolids-N/ha was applied at Solano, and 6±1 Mg biosolid-N/ha was applied at Merced. Our estimates of cumulative biosolids-N applied using results from the fresh biosolids data are accurate with historical records (1995-2011) of how much biosolids-N was applied to the fields by 1%, giving us confidence in our estimates of cumulative C applied.

3.4.2. Soil pH and texture

Soils at the Sacramento and Solano sites were predominately clay loam, while soils at the Merced site were sandy loam (Supplemental Table 1). Surface (0-10 cm) soil pH varied widely among the sites, ranging from 6±0.1 (Solano) to 8±0.2 (Merced) (Supplemental Material: Table 1). Across all sites, soil pH was lower in the surface soil and increased with depth; suggesting that the decomposition of biosolids may be the cause of lower pH due to the release of organic acids to the soil. There was no significant treatment difference in soil pH at Sacramento ($p = 0.4$) or Merced ($p=0.1$). There was a marginally significant treatment effect at Solano ($p=0.1$), where soils amended with biosolids had a pH of 7.0 compared to 6.6 in control soils.

3.4.3. Soil carbon stocks

Percent C in the biosolids amended and control soils were significantly different from each other in the Sacramento and Solano sites down to 100 cm depths (both $p<0.001$); however, no significant differences were observed between treatments in the Merced soils ($p=0.6$). Solano site experienced the greatest increase in SOC with biosolids amendment. At this site, cumulative SOC to 100 cm in biosolids-amended transects were 10±1 Mg C/ha greater than control soils. Carbon stocks in biosolids-amended soils at the Sacramento site were approximately 5±2 Mg C/ha greater than control soils. The lowest amount of C sequestered was at the Merced site, with 3±3 Mg C/ha more in the biosolids-amended soils (Figure 1a).

At each site, an average of 77%, 89%, and 80% of cumulative soil C to 100 cm occurred in the top 30 cm of amended soil in Sacramento, Solano, and Merced respectively. The greatest relative increase in SOC tended to occur in the top 30 cm of soil due to biosolids application. The application of biosolids significantly increased SOC content at the Solano site by 50% from 0-10 cm ($p<0.001$; Figure 1a). Biosolids application marginally increased SOC content of the 0-10 cm depth compared to the control soils at the Sacramento ($p= 0.10$) and Merced ($p=0.1$) sites, and no significant differences were detected from 10-30 cm at any site.

At the Solano site, biosolids application significantly increased cumulative SOC by 50% ($p<0.001$), with significant treatment effects observed in the 0-10 ($p<0.001$), 30-50 ($p<0.001$), 50-75 ($p=0.004$), and 75-100 ($p=0.001$) depth intervals. Cumulative SOC to 100 cm in biosolids amended soils were 32% and 20% greater compared to controls in the Sacramento and Merced sites though not statistically significant. No depth increments from 30-50 cm, 50-75 cm, or 75-100 cm were significant at the Sacramento or Merced site.

Soil inorganic C (SIC) was approximately 22% of the total soil C across both treatments in the Merced site. Soil inorganic C in the unamended controls ranged from 0-120 Mg SIC/ha. Biosolids amended soils had SIC contents ranging from 0-104 Mg C/ha. The wide ranges in SIC content suggest high spatial variability of the soil conditions at this site. There was no significant treatment effect on SIC between the biosolids amended and unamended soils ($p=0.2$), therefore changes in SIC were not included in estimates of C sequestration per area or per amount of biosolids applied.

3.4.4. Rates of C sequestration

Rate of SOC sequestration was calculated for each transect based on changes in soil C per area per year to a 30 cm and 100 cm depth and based on the amount of biosolids applied. The highest rate of SOC sequestration occurred at the Solano site, regardless of the approach used to determine C sequestration rates. However, rates of soil C sequestration were higher at the Merced site than the Sacramento site when expressed as a function of mass of biosolids applied, but highest at the Sacramento site compared to the Merced site when expressed on an areal basis (i.e. Mg C/ha; area of field sites) from 0-30 cm. If we assume linear rates of C sequestration over the 20-y period, annual rates of C sequestration to a 30 cm depth were approximately 0.4, 0.3, and -0.04 Mg C ha^{-1} y^{-1} at the Solano, Sacramento, and Merced sites, respectively (Table 2). Negative values indicate less C at the 0-30 cm depth in the biosolids treated transects compared to the control transects. When considering a 0-100 cm soil depth, these values increase to 0.5 (Solano) and 0.2 (Merced) Mg C ha^{-1} y^{-1} except in Sacramento where C rate was estimated to be 0.2 Mg C ha^{-1} y^{-1}. When expressed as changes in mass of soil C per mass of biosolids applied, average C sequestration rates were 1±0.2 Solano, 0.04±0.1 in Sacramento, and 0.5±0.4 in Merced. These rates were marginally significant from 0 at the 0–100 cm depth across all sites ($p = 0.10$).

3.4.5. Soil total N

In the top 10 cm, soil total N increased from 1±0.1 Mg N ha^{-1} in the control to 2±0.2 Mg N ha^{-1} in the biosolids amended soil in the Solano site ($p<0.001$), and from 1±0.1 Mg N ha^{-1} to 3±0.3 Mg N ha^{-1} in the Sacramento site ($p<0.001$; Figure 1b). The Merced site experienced no significant changes in soil total N from 0-10 cm ($p=0.3$). Total soil N increased from 2±0.1 Mg N ha^{-1} in the control to 4±0.2 Mg N ha^{-1} in the amended soils at the Sacramento site ($p<0.001$) and from 2±0.1 Mg N ha^{-1} in the control transects to 3±0.1 Mg N ha^{-1} in biosolids amended soils in Solano ($p<0.001$) from 0-30 cm. Soil total N to 30 cm at the Merced site was greater in amended soils (4±0.4 Mg N ha^{-1}) compared to control soils (3±0.1 Mg N ha^{-1}), this change was not statistically significant ($p=0.3$). Down to 100 cm depth, soil total N increased from 4±0.1 Mg N ha^{-1} to 5±0.2 Mg N ha^{-1} ($p=0.02$) in Sacramento and from 3±0.1 Mg N ha^{-1} to 4±0.1 Mg N ha^{-1} ($p<0.001$) in Solano. Similarly, the application of biosolids increased total N in Merced soils by 35% though marginally significant (5±0.2 Mg N ha^{-1} biosolids; 4±0.1 Mg N ha^{-1} control) from 0-100 cm ($p=0.06$).

3.4.6. Microbial biomass

At the Sacramento site, MBC from 0-10 cm in biosolids amended soils was 656±44 mg C kg^{-1} compared to 280±41 mg C kg^{-1} in the control (Figure 2a; $p<0.001$). Biosolids amended soils from 10-30 cm in the Sacramento site also increased from 66±8 mg C kg^{-1} to 150±15 mg C kg^{-1} ($p<0.001$). At the Solano site, microbial biomass C increased in amended soils to 378±60 mg C kg^{-1} from 234±37 mg C kg^{-1} in the 0-10 cm depth ($p=0.2$) and to 163±40 mg C kg^{-1} from 48±7 mg C kg^{-1} in 10-30 cm depths ($p=0.02$). The ratio of microbial biomass to SOC (MBC:SOC) were significantly higher in amended soils at the Solano site ($p=0.01$), and not significant at the Sacramento ($p=0.2$) and Merced site ($p=0.1$; Figure 2b).

Trends in MBN followed the similar patterns as MBC. Biosolids application significantly increased MBN compared to the control by 79% from 0-10 cm and 114% in the 10-30 cm soil in the Sacramento site (both $p<0.01$). No increase was observed in microbial biomass N at from 0-10 cm and 0-30 cm at the Solano site ($p=0.4$; 0.3). Significant treatment effects in MBN were not observed at either depth at the Merced site ($p=0.1$; Figure 2c).

3-5. Discussion

3.5.1. Consistency of biosolids characteristics

Biosolids are stabilized and often dewatered prior to land application using a variety of technologies, such as lime stabilization, anaerobic digestion, or composting (Wang et al., 2008). The type of stabilization and dewatering technologies used determines the physical and chemical composition of biosolids, which can vary considerably (Wang et al., 2008). The extent to which biosolids effect soil C sequestration, nutrient cycling, and plant uptake may be dependent on its chemical and physical composition (Mekki et al., 2019; Nicolás et al., 2014). One study found that non-composted biosolids promoted OC protection in microaggregates; whereas, composted biosolids promoted OC protection as coarse particulate organic matter within macroaggregates suggesting that composition plays a role in the physical incorporation of C in soils (Nicolás et al., 2014). The biosolids applied in all three sites were generated from anaerobic digestion of wastewater and came from wastewater treatment plants that supply biosolids within the study region, although the size and management conditions of the plants varied widely. The elemental composition of the biosolids used in this study were similar to those reported in other studies in different regions throughout the world for anaerobically digested biosolids (e.g., Fernandes et al., 2005; Hamdi et al., 2019; Petersen et al., 2003). These findings suggest that biosolids quality varied little in the study region due to common stabilization technologies, and differences in soil responses in our study are not likely attributed to differences in biosolids amendments. However, it is possible that biosolids chemical composition varied over the course of the 20 years of application.

3.5.2. Biosolids effects on soil organic C stocks

Amendments are a direct source of C, some of this C may persist in soils, while some is lost through decomposition and respiration (Ryals et al. 2014). In our study, long-term (20-year) biosolids application increased SOC in the top 30 cm of soils in three agroecosystems in California. This finding is consistent with studies in other regions that have documented beneficial reuse of biosolids as a strategy to sequester C in soils in surface soils (Brown et al., 2011; Hamdi et al., 2019; Nicolás et al., 2014; Pan et al., 2017). There are number of factors that improving SOC in soils including improvements in soil quality, agronomic productivity, and the enhancement in soil microbial diversity (Lal, 2014). Although there is not one direct measurement of soil quality, one must infer through many soil characteristics and parameters (Mukherjee and Lal, 2014). Soil C being one of the most important and interrelated parameter that serves as the basis for some of the most important soil processes such as N and P cycling, but also as a indicator for a soils's resilience to climate change and management practices (Lal, 2014).

Assuming initial concentrations of C in the control soils were similar to the biosolids amended before biosolids were applied 20 years ago, soil stocks from 0-100 cm were: 23±3MgC/ha at the Sacramento site; 25±2 MgC/ha at the Solano site and 28±2 MgC/ha at the Merced site. From 0-100 cm the biosolids treatment soil C was 27±4 MgC/ha at Sacramento; Solano is 36±2 MgC/ha; and Merced was 36±2 MgC/ha. The significant change from the control soil C to the biosolids treated soil was in the Solano site indicating that the rate of C sequestration there is faster or soil properties and management may offer more favorable environments for increasing soil C (Poulton et al., 2018). There is little variation in soil C concentration across sites in the control soils, suggesting all soils started at the same relative soil C content.

The largest increase and changes in soil C from biosolids application was found at the Solano site. This was surprising, as this site received the least cumulative amount of biosolids applied over the 20-year period. Climatic conditions and soil textures are similar at these two sites, but the Sacramento site is flood irrigated annually between May and November (U.S. Climate Data). We expected to see the largest increase in SOC at this site due to the high rate and frequency of biosolids application. Cumulative biosolids application was nearly five-fold greater at the Sacramento site compared to the Solano site. Despite intensive biosolids use at Sacramento, increases in SOC to 100 cm (5±2 MgC/ha) were about half of the observed changes at the Solano site. Further, increases in SOC were restricted to the top 10 cm of soil, while no significant differences were observed in any depth interval from 10 to 100 cm. Flood irrigation can cause a pulse of carbon dioxide from microbes which consequently causes C losses (Guo et al., 2017). While the net balance of C gains to C losses at the Sacramento site remained positive, flood irrigation may have prevented further buildup of soil C.

The Merced site was flood irrigated and managed for feed crop production with an alfalfa-corn rotation, resulting in frequent physical disturbance of surface soils with coarser soil texture. Tillage of soils commonly breaks up soil structure and reduces aggregation, resulting in a net loss of soil C in surface soils (Stewart et al., 2012); however, increases in C in deeper soil layers are sometimes observed in tillage systems (Spaccini and Piccolo, 2019). One of the paired transect was classified as a Mollisol, which is known to be a soil of higher organic matter in the surface, no large variation in SOC was observed from 0-30 cm in the controls soils compared to the other 2 paired transects which were classified as Alfisols (Mollisol: 18±1 Mg C ha^{-1}; Alfisols: 19±1 and 15±1 Mg C ha^{-1}). This suggests that losses of SOC from the Mollisol was most likely due to agricultural practices such as tillage and flood irrigation. We observed no treatment difference in SOC to the 30 cm at this site but there were significant increases in SOC in depth intervals from 30 to 100 cm. These observations may be explained by movement of C below 30 cm through the soil profile caused by a combination of coarse soil texture, tillage, and flood irrigation practices as Spargo et al. (2008) also observed in their field sites that have had biosolids applied to no-till field sites in Virginia. Flood irrigation in soils with high salinity may cause the desorption and transportation of dissolved SOC to deeper soil layers, leading to lower levels of C (Lu et al., 2020; Mavi et al., 2012). Less frequent and lower application rates of biosolids can also inhibit accumulation of organic C (Hamdi et al., 2019).

3.5.3 Rates of soil C sequestration

The amount of organic matter amendments added to a soil can influence the net soil C sequestration in agroecosystems. Our study sites included a range of application rates and frequencies, resulting in net application of biosolids over 20 years of 0.5 Mg biosolids-C/ha (Merced), 1 Mg biosolids-C/ha (Solano), and 0.04 Mg biosolids-C/ha (Sacramento). A review of field studies determined that rates of C sequestration from biosolids application ranged from 0.014 to 0.54 Mg C per Mg dry biosolids, considering a 0-15 cm soil depth and no change from 15-30 cm (Brown et al., 2011). A more recent study found rates as high as 1 Mg C per Mg dry biosolids in the top 10 cm of soil (Pan et al. 2018). There is a lack of data on rates of SOC changes with biosolids application in soils below 30 cm obscuring the potential for long-term C storage in deeper soil.

Carbon benefits from biosolids varied widely within and between sites in this study. Rates of C sequestration were highest at the Solano site. Notably, the rate of SOC sequestration at Solano, 1 Mg C per Mg dry biosolids applied, is greater than other published values (Brown et al. 2011, Pan et al. 2018). Both Sacramento and Solano sites are annual grasslands managed for rotational cattle grazing, but Sacramento is flood irrigated and Solano is rainfed. Frequent flood irrigation is used at the Sacramento site, while the Solano site is rainfed. These factors may also contribute to C losses from the amended soils through leaching and mineralization of C following an irrigation event (Guo et al., 2017).

Slightly negative values were observed in three of the nine paired transects indicating net soil C loss in amended plots compared to controls. Two of these negative rates were at the Merced site, which had the lowest frequency and relatively low rates of biosolids application. In contrast to the other two sites, cultivation practices at the Merced site were also more intensive which could have resulted in faster rates of decomposition of biosolids-C. The Merced site also had coarse soil texture, which is generally associated with lower SOC. One of the paired transect was classified as a Mollisol, which tend to have higher SOC content compared to Alfisols, the other soil order found at this site. SOC content in controls soils within this site were not significantly different (Mollisol:

18±1 Mg C ha^{-1}; Alfisols: 19±1 and 15±1 Mg C ha^{-1} in the 0-30 cm depth). However, a coarse soil texture does not preclude a positive impact of biosolids amendments on soil C, and there is evidence that repeated application of biosolids lead to sustained increased in SOC in croplands with coarser soil texture (Hamdi et al., 2019).

Our results demonstrate that the effect of biosolids is not restricted to the shallow depth of application and incorporation, and that increases in SOC can our considerably below 30 cm across all our transects. Positive soil C sequestration rates were, on average, 85% greater when accounting for C changes to 100 cm depth, but ranged from 2% to 254% greater. Current assessments of biosolids amended soil are likely underestimating C storage; thus, misinterpreting the soils climate change mitigation potential. Further, we currently have little understanding of the mechanisms driving SOC changes due to organic matter amendments at lower depths, and a lack of estimated turnover times of this C pool (Gravuer et al., 2019).

3.5.4. Biosolids effects on soil total N stocks

Biosolids contain abundant quantities of organic N which is not immediately available for plant uptake but can remain in the soil and slowly mineralize to plant available forms (Nicholson et al., 2018). Biosolids applications at the Merced site did not significantly increase in total N compared to the controls, and the lack of significance may be driven by a high level of variation. One explanation is the difference in soil texture among the sites. The coarse soil texture and flood irrigation at the Merced site can contribute to high rates of N leaching and denitrification (Weil and Brady, 2017). However, a previous field study found that after three years of applying biosolids at different rates yearly, total N increased in a rainfed irrigated sandy soil and a sandy loam soil (Hamdi et al., 2019). The Merced site was flood irrigated, had no manure additions from cattle grazing, grew corn which has a high N uptake, suggesting irrigation practices influences total N retention in soil instead of soil texture.

Nicolás et al (2014) and Pan et al. (2018) concluded that application rate may be the reason they found an increase in total N in their studies compared to the controls. Both the Sacramento and Solano sites had significant increases in total N to 100 cm which is possibly connected to similar soil texture and higher application rates. The lack of treatment difference in soil total N below 10 cm at the Merced site can be attributed to low frequency of biosolids application and rates, coarser soil texture, and management.

3.5.5. Microbial biomass C and N

Low application rate, soil texture, or management practices possibly contributed to no change in MBC at the Merced site. However, a study by Speir and collaborators (2004) show an increase of microbial biomass C in the top 10 cm of soil in ryegrass-clover pasture with the soil texture as the Merced site after only four years of annual applications. Other studies have shown an increase in MBC when rate of biosolids application is increased indicating application rate determines changes in microbial activity (Fernandes et al., 2005; Hamdi et al., 2019).

MBC:SOC percentages from highest to lowest were found in Solano, Sacramento, and Merced, coinciding with changes in SOC stocks. Sacramento and Solano had similar MBC:SOC percentages in the controls suggesting that soil texture influenced similar result between both agroecosystems. Low application rates, coarse soil texture, and high IC may contribute to unfavorable environments for microbes (Mavi et al., 2012).

Microbial biomass N is a good measure of mineralizable N since it is dependent on microbiologically mediated processes (Myrold, 1987; Soon et al., 2007). Biosolids have been shown to increase MBN in previous studies (Bai et al., 2019; Sánchez-Monedero et al., 2004; Santos et al., 2011; Speir et al., 2004).

3-6. Conclusions

The current results show that despite high yearly application rates, C sequestration at the Sacramento site was the second highest between all three agricultural ecosystems, implying that management may also play an important role on C storage. We found the greatest increase in SOC from biosolids application at the Solano site. This site received low application rates and frequencies of biosolids, yet C sequestration rates were highest here. This is true whether considering C sequestration on an area basis or as a function of the amount of biosolids applied. Surprisingly, the site with the highest frequency and rates of application had the lowest C sequestration rate, indicating that other factors influences C storage or the potential to store additional soil C has been reached. Additionally, we have shown that not accounting for deeper SOC below 30 cm depths can lead to an underestimation of C sequestration as shown at the Solano site (estimated C sequestration rate 0.53 Mg C ha^{-1} y^{-1} 0-100 cm; 0.40 Mg C ha^{-1} y^{-1} 0-30 cm). Further application of biosolids at these sites may reveal further information about the ability of the soils to sequester C and the extent of which a soil can be enriched by C or will it reach a saturation point. In addition, determining where C is in different pools, assessing other soil physicochemical parameters, such as inorganic binding agents (aluminum and iron oxides, cation bridging) may give better insight to the stability of C. Assessing the effects of biosolids application and other management practices on C and N cycling, and mechanistic studies is needed to determine optimal agricultural context for biosolids reuse.

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Conflict of Interest
The authors declare no conflict of interest.

3.7 References

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3.8 Tables

Table 3-1. Site characteristics and biosolids application information.

Site	Transect #	Frequency of Application	Total Amount Applied from T=0 Mg Dry Biosolids/ha	Estimated C Applied (Mg/ha)	N Applied (Mg/ha)	pH (0-30 cm)	Soil Texture	MAT °C	MAP mm
Sacramento	1	Yearly	298	103	16	5.9±0.3	Clay Loam	16	460
	2	Yearly	420	145	22				
	3	Yearly	361	125	19				
Solano	4	Every 3 Years	82	29	4	5.9±0.2	Clay Loam	23	630
	5	Every 3 Years	86	30	5				
	6	Every 3 Years	53	18	3				
Merced	7	Every 5 Years	157	54	8	8.0±0.1	Sandy Loam	18	250
	8	Every 5 Years	65	23	4				
	9	Every 5 Years	93	32	5				

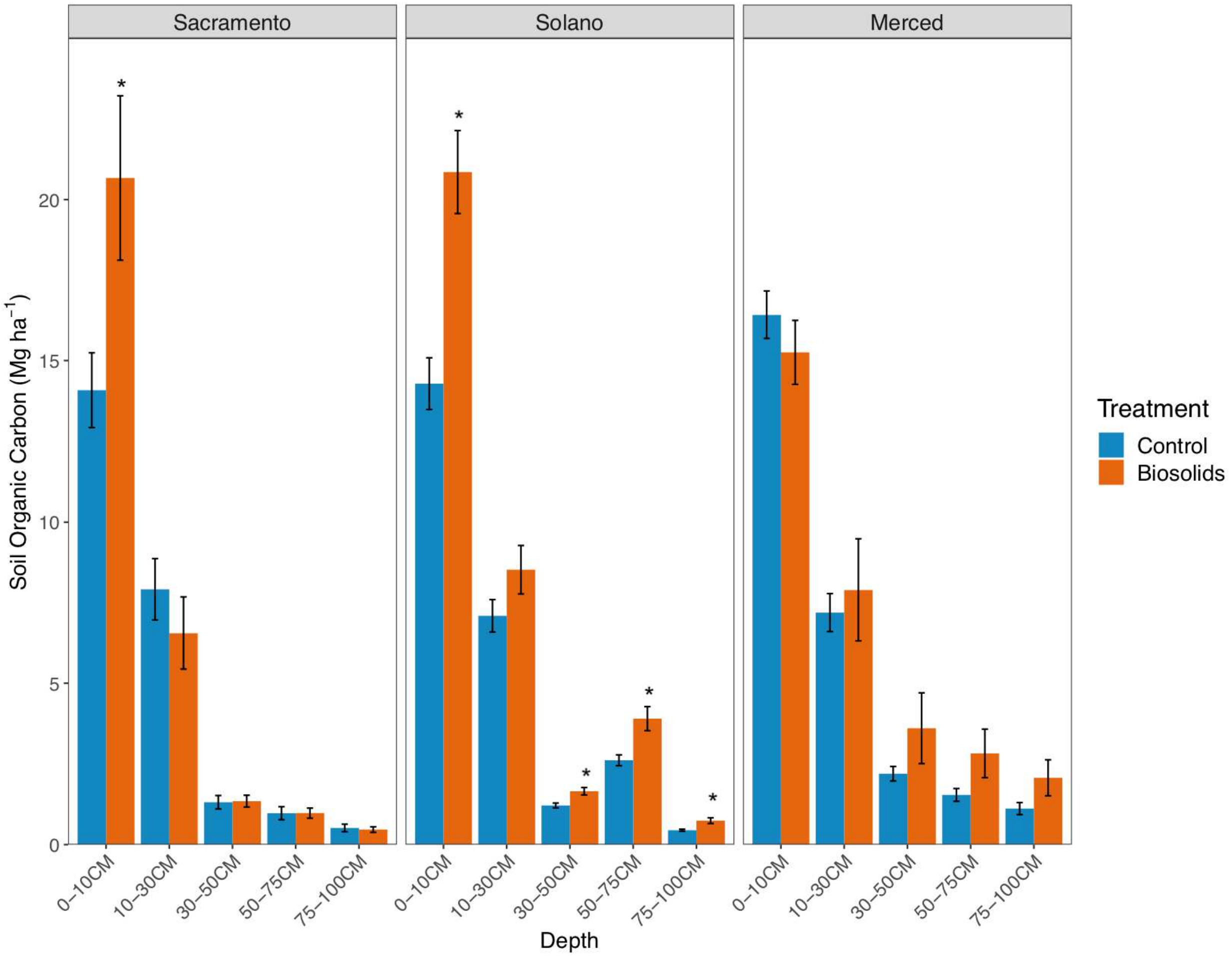
Table 3-2. Sequestration rates using 3 methods: 0-30 cm, 0-100 cm, and Mg C stored per amount applied for each site. Values are site means ± standard errors, number of samples per type of sequestration rate was 90.

Site	Rate of C storage 0-30 cm (Mg C $ha^{-1}yr^{-1}$)	Rate of C storage 0-100 cm (Mg C $ha^{-1}yr^{-1}$)	Per Biosolids Applied (0-100 cm) (Mg C / Mg biosolids)
Sacramento	0.3±0.2	0.2±0.2	0.0±0.1
Solano	0.4±0.1	0.5±0.1	1.±0.2
Merced	-0.0±0.2	0.2±0.2	0.5±0.4

3.9 Figures

Figure 3-1. Mean values in (a) soil organic C and (b) soil total N after 20 years of biosolids application in five depth increments to 100 cm in three agricultural ecosystems. Asterisks indicate statistical significance between treatments ($p<0.05$). The error bars are standard errors.

a).



b).

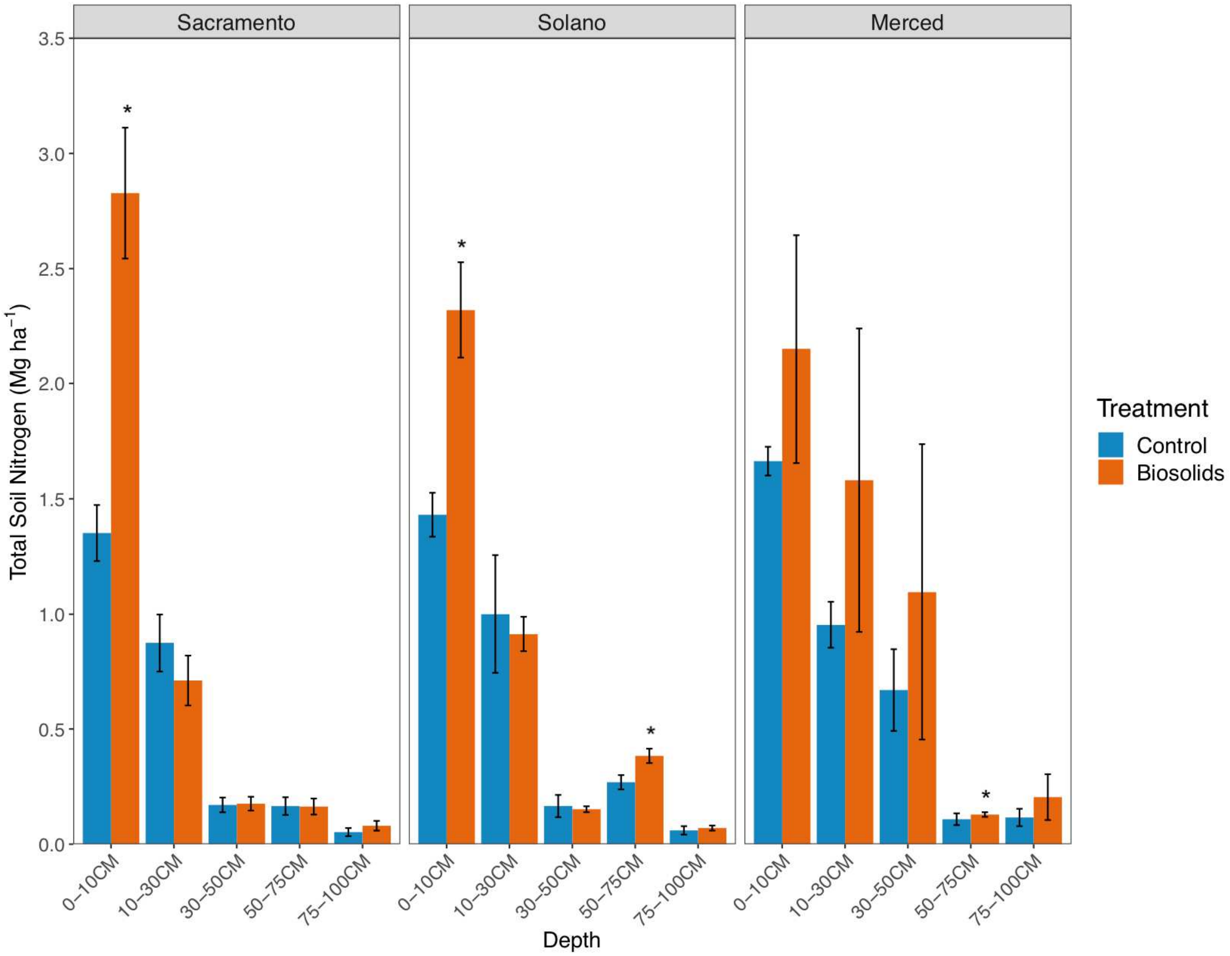
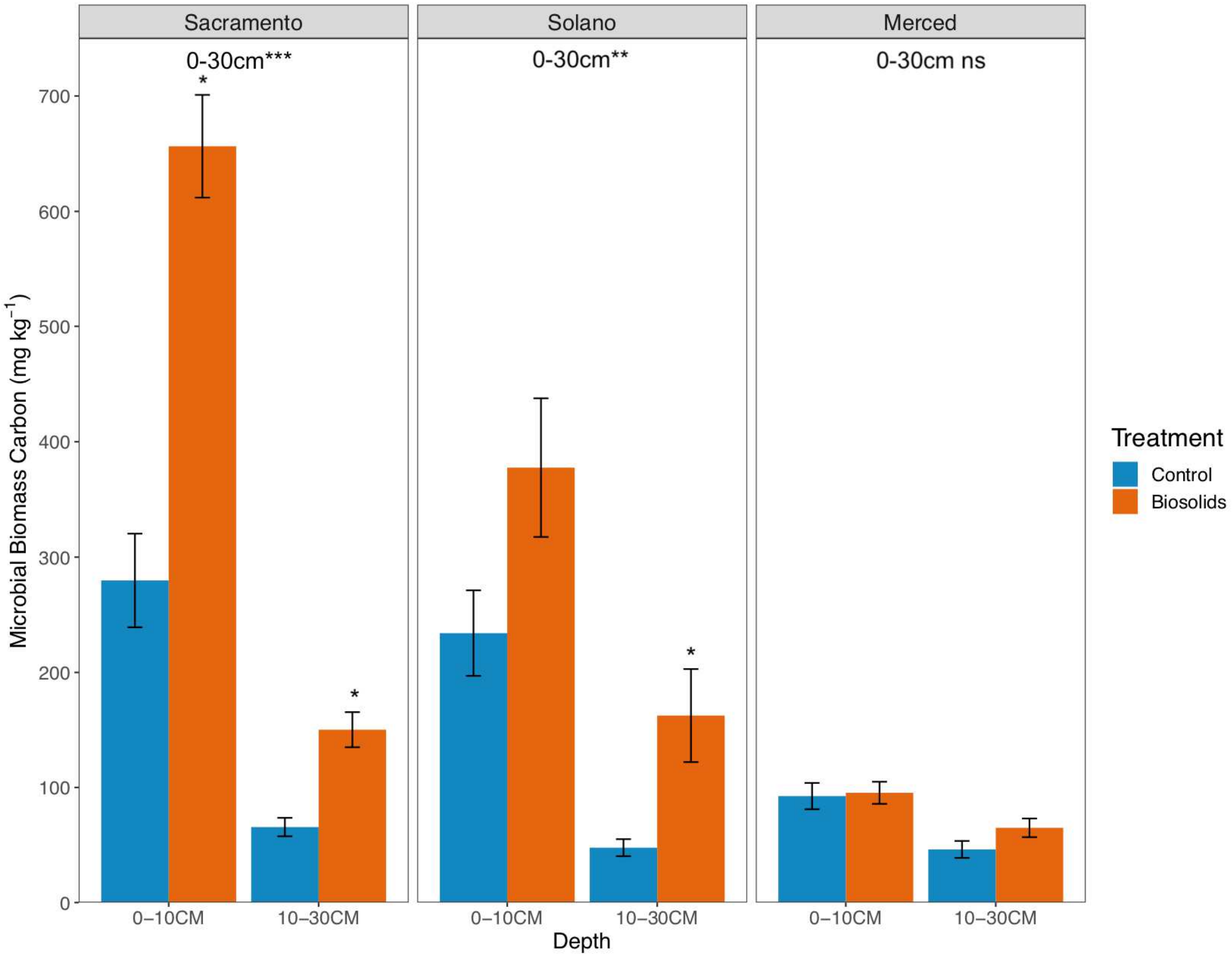
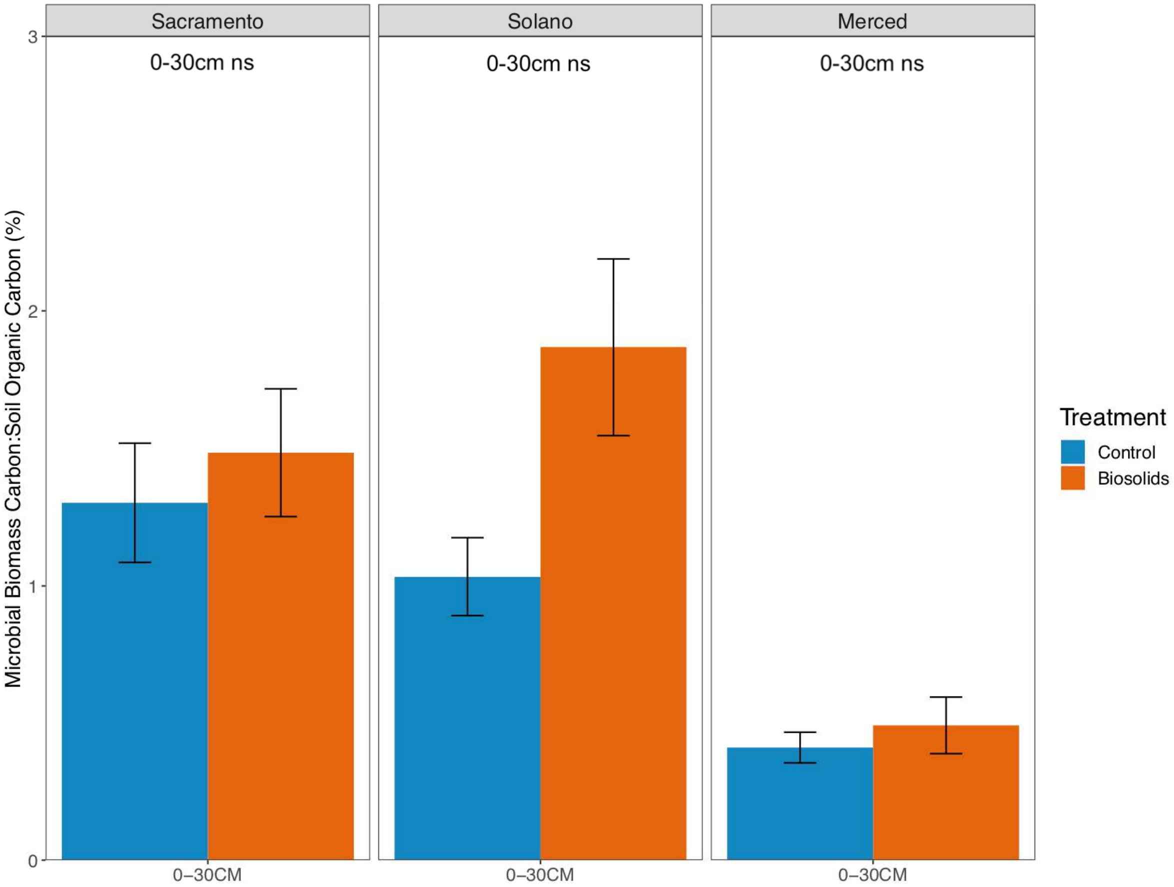

Sacramento
Solano
Merced
Total Soil Nitrogen (Mg ha^{-1})
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
*
*
*
0–10CM
10–30CM
30–50CM
50–75CM
75–100CM
Depth
Treatment
🟦 Control
🟧 Biosolids

Figure 3-2. Changes in microbial biomass a). C; b). Microbial biomass carbon to soil organic carbon ratio percentages; c) and microbial biomass N separated by depth and down to 30 cm. Asterisks indicate statistical significance (* p<0.05; **p<0.01; ***p<0.001; ns p>0.05).

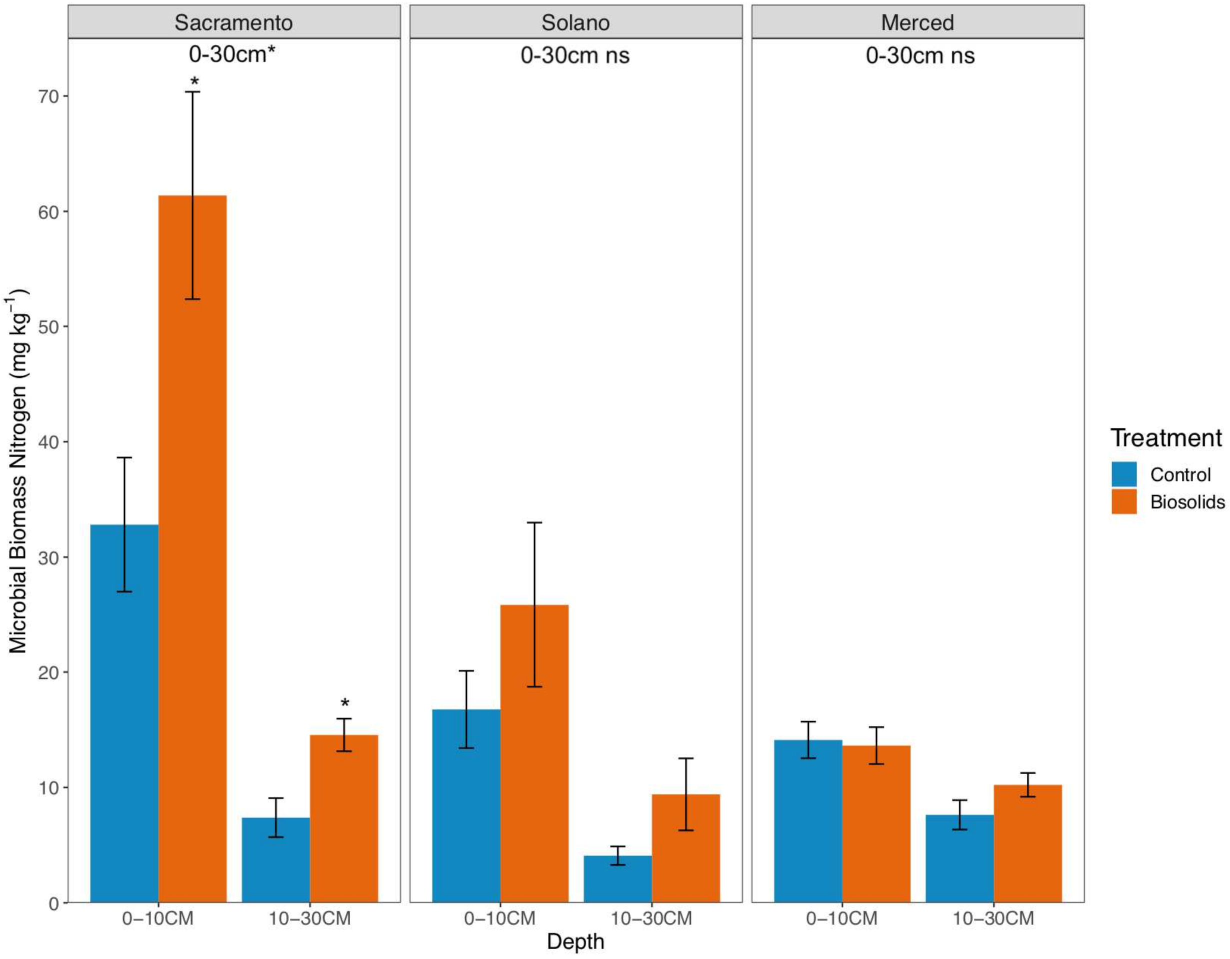
a).



b).


Sacramento
Solano
Merced
0-30cm ns
0-30cm ns
0-30cm ns
Microbial Biomass Carbon:Soil Organic Carbon (%)
3
2
1
0
0–30CM
0–30CM
0–30CM
Treatment
Control
Biosolids

c).


Sacramento
Solano
Merced
0-30cm*
0-30cm ns
0-30cm ns
*
*
Microbial Biomass Nitrogen (mg kg^{-1})
70
60
50
40
30
20
10
0
0–10CM
10–30CM
0–10CM
10–30CM
0–10CM
10–30CM
Depth
Treatment
Control
Biosolids

4. Carbon persistence in long-term amended biosolids soils in California agroecosystems

4.1 Abstract

Biosolids are capable of building total soil organic matter, but their ability to increase carbon (C) and nitrogen (N) in stable fractions in deeper soil depths is less understood. Our aim was to assess the mechanisms that influence soil C and N dynamics in biosolids amended soils from three sites (Sacramento, Solano, and Merced). We used density fractionation techniques (0-10 cm; 30-50 cm; 75-100 cm), organo-mineral extractions (0-10 cm; 30-50 cm; 75-100 cm), and correlations between Ca^{2+} and SOC (Merced: 0-10 cm, 30-50 cm, 75-100 cm), in agricultural ecosystems with different site conditions, management, and application rates and frequencies. Density fractionations showed that soils amended with biosolids had more C and N content associated to the free- and occluded light fractions relative to the control from 0-100 cm in the Sacramento and Solano sites; however, the Merced site had a greater relative increase of C and N associated to the heavy fraction down to 100 cm. Effect sizes show that biosolids application increases C and N content in the light fractions in shallower depths and in both light and heavy fractions at the 75-100 cm depth. Ratios of C to iron (Fe) and aluminum (Al) show that chelation is an important mechanism of C stabilization in Sacramento and Solano down to 100 cm. No (0-10 cm) to negative (75-100 cm) correlations were observed between Ca^{2+} and SOC in the amended soils in Merced, indicating that the biosolids are influencing how SOC adsorbs in the soil. Our results indicate that long-term application of biosolids can sequester C in labile and physically protected pools down to 100 cm depths and contributes to climate change mitigation.

4.2. Introduction

There has been growing interest in soil carbon (C) sequestration as a way to reduce atmospheric carbon dioxide (CO_2) and mitigate climate change (Griscom et al., 2017; Minasny et al., 2017; Roe et al., 2019; Torri et al., 2014). Soils contain the largest terrestrial C pool, storing approximately 1580 Gt of organic C in the top meter of soil (IPCC, 2019). Hence, soil is considered to be a major sink for C storage because of its potential to sequester and retain large amounts of C (Che et al., 2021). Management-induced soil C sequestration has been studied in agricultural contexts including conservation tillage, improved grazing, fertilizer use efficiency, cover cropping, and organic matter amendment applications (Brar et al., 2013; Buckley Biggs and Huntsinger, 2021; Deen and Kataki, 2003; Mazzoncini et al., 2011; Powlson et al., 2012; Ryals et al., 2014). Organic matter amendment application has been proposed as a way to increase soil organic carbon (SOC) stocks through C inputs, and indirectly through the promotion of plant production (Ryals et al., 2014). However, not all organic amendments have the same effects on soil C dynamics. Differences in organic material, treatment processes, and management strategies can influence soil biogeochemical cycling; thus, influencing the affects the magnitude of changes to soil organic matter (SOM) and the potential benefit of this practice.

The production of biosolids has increased in the last decades, and their accumulation and disposal pose environmental challenges (Kominko et al., 2017; Torri et al., 2014). Hence, a lot of attention has been paid to recycling biosolids and using them as a soil organic amendment due to its valuable source of plant nutrients (Brown et al., 2011; Kominko et al., 2017). Long-term application of biosolids to agroecosystems has been shown to increase soil organic C which in turn increases improves soil fertility (Brown et al., 2011; Charlton et al., 2016; Cogger et al., 2013; F. Nicholson et al., 2018; Villa and Ryals, 2021). Although long-term biosolids application has been shown to provide benefits for soil fertility and increasing soil organic C content, little is known about the persistence of C in agroecosystems soils, particularly in deep soils (Tian et al., 2009; Torri et al., 2014). Some studies have emphasized the importance of sampling deeper depths due to the nature of subsoils having larger concentrations of clays and organo-mineral complexes that can contribute to the persistence of soil C over long periods of time (Kögel□Knabner et al., 2008; Poeplau and Don, 2015; Tautges et al., 2019; Villa and Ryals, 2021; Yost and Hartemink, 2020). Existing studies that assessed the persistence of C in different pools, using fractionation techniques, only covered the top 30 cm of soil (Chiu and Tian, 2011; Pan et al., 2017; Silva et al., 2015; Wijesekara et al., 2017). So far, limited studies have sampled down to 100 cm and determined the persistence of C pools from soils that have received biosolids application (Wang et al., 2021). Thus, there is little information exists on how biosolids application may influence soil C sequestration or the persistence of the C once it enters the soil system. Improved understanding of the mechanisms responsible for SOC stabilization in biosolid amended soils is critical for providing insight on the vulnerability of the sequestered soil C, and its implications for improving soil fertility and climate benefits (Tautges et al., 2019; Wang et al., 2021).

The rate at which SOC is mineralized to the atmosphere is determined by soil physiochemical properties that determine stability and stabilization mechanisms of soil C; such as occlusion of organic C within aggregates, sorption to mineral surfaces, and co-precipitation of organics with dissolved metals (Heckman et al., 2018). Soil fractionation techniques can provide ecologically-relevant data on distribution of soil C and N in pools that cycle at different rates, depending on the accessibility of the C in each pool to microbes (Powlson et al., 2012; Ryals et al., 2014; Swanston et al., 2005). For this study, bulk soil C and N was partitioned into three fractions using a physical, density fractionation procedure (Ryals et al., 2014; Swanston et al., 2005). These fractions are the free-light fraction (FLF), the physically protected occluded-light fraction (OLF), and the heavy fraction (HF). Each of these fractions provides information on the potential turnover rates of C and N in soils. The organic matter in the FLF closely resembles plant material which is "free floating" in the soil matrix and not protected by association with soil minerals. The unprotected SOC in the FLF is relatively more easily mineralizable by microbes and often has the fastest turnover time (Marín-Spiotta et al., 2008; Swanston et al., 2005). The OLF contains C and N that is physically protected by occlusion inside soil aggregates. This makes it more difficult for decomposers as microbial access to the substrate is limited; hence, this fraction tends to have a slower turnover rate than that of FLF. The HF contains C and N that is chemically bound to mineral surfaces and typically has the longest residence time compared to FLF and OLF.

Previously, it was assumed that storage and stability of SOC can be predicted from soil's clay content, but recently, it was shown that other physico-chemical parameters may be better predictors (Rasmussen et al., 2018). Studies have indicated that SOC content may be related to extractable metals and exchangeable cations emphasizing that clay content is not always correlated with persistence of SOC (Kaiser and Guggenberger, 2000; Rasmussen et al., 2006). There have been advances in showing that polyvalent cations, such as calcium (Ca^{2+}), plays a significant role in the stabilization of SOC by creating cation bridges between organic compounds and mineral surfaces (Rasmussen et al., 2018; Rowley et al., 2021; Solly et al., 2020). In addition, Ca^{2+} can indirectly contribute to SOC persistence as it is contributes to soil aggregation, and thereby SOC stabilization in OLF (Oades, 1988; Rowley et al., 2021, 2018). Biosolids contain high levels of iron (Fe) and aluminum (Al), and studies have shown that long-term applications of biosolids could increase soil amorphous Fe and Al, leading to increased degree of SOC stability (Chen et al., 2019; Chiu and Tian, 2011; Silva et al., 2015; Tian et al., 2013; Wang et al., 2021). The properties of these organic materials, such as nutrient composition, may raise some additional questions and challenges on how to properly manage them. Hence, it is important to understand how biosolids influence C persistence in agricultural soils with different soil physicochemical properties, such as soils that are coarser in texture, or in soils with higher concentrations of Ca^{2+} present.

We determined the changes in the amount and distribution of C in soil fractions from three agroecosystems that have received twenty-years of biosolids application. We assessed these changes in the surface soil (0-10 cm), as well as in subsoil (30-50 cm and 75-100 cm). We also evaluated potential mechanisms that influence soil C dynamics across these fractions, including organo-mineral associations at all sites (referred to as Merced, Sacramento, Solano) and Ca^{2+}concentrations in the HF at the site with high pH (Merced). We hypothesized that: 1) Biosolids will increase C and N present in the FLF in surface soil due to direct application of biosolids C and N across the three sites; 2) Positive treatment effects on C and N will be dependent on fractions and depth; 3) Both amended and control soils from sites that have a lower pH will have stronger organo-mineral C content than the site with a high pH; 4) At the Merced site, Ca^{2+} concentration in the HF will be the primary stabilization mechanism for C regardless of treatment due to the high concentrations of calcium carbonate ($CaCO_3$).

4.3 Materials and Methods

4.3.1 Study sites and experimental design

The study sites were situated in Northern California, including locations in Sacramento County, CA (38°20'06.3"N, 121°10'06.5"W), Solano County, CA (38°11'52.3"N, 121°45'38.0W), and Merced County, CA (37°04'20.3"N, 120°31'43.0"W). Sampling at these sites consisted of establishing an unamended control and an amendment treatment where biosolids were applied for twenty years as described in detail in Villa and Ryals. (2021). Sampling was replicated at three fields within each of the three locations (n=9). Soil samples were collected using a 57 mm-diameter auger at five depth increments, 0-10 cm, 10-30 cm, 30-50 cm, 50-75 cm, and 75-100 cm, at every ten meters along a 100-m transect (n=10). Transect starting points and bearings were randomly selected from within each field, excluding a 5 m buffer from edges. A total of 900 soil samples were collected and transported to University of California (UC) Merced for laboratory analysis. Total organic C and N concentrations were measured for all 900 samples as described in Villa & Ryals (2021). One paired transect (amended treatment and an unamended control) with the highest relative change between the pair was selected per site for density fractionations resulting in one paired transect per site (n= 3).

The Sacramento site consisted of flood-irrigated annual grasslands managed for grazing beef cattle. Livestock was rotated every 60-70 days depending on feed availability. Soils are classified as Alfisols from the Hicksville and Corning series. The Solano site consists of rainfed annual grasslands used for grazing lambs and beef cattle. Grazing intensity is 0.3 animal units ha^{-1}. Livestock are rotated seasonally and based on feed availability. Soils are classified as Alfisols from the Antioch and Pescadero series and Vertisols from the Altamont soil series. The Merced site is flood irrigated and managed for livestock feed crops consisting of 1 y corn and 4 y alfalfa rotations for livestock silage and were tilled after each rotation. Soils are classified as Mollisols from the Pozo series and Alfisols from the Fresno series (Table 3-1).

4.3.2. Density fractionations

Soils from each paired transect and from depths 0-10 cm, 30-50 cm, and 75-100 cm were density separated at 1.85 g cm^{-3} as described in Swanston et al. (2005). Briefly, 20 g of soil was gently mixed with 75 ml of sodium polytungstate (SPT). After centrifuging for one hour at 3,500 rpm, the FLF was floated off, filtered and rinsed with DI water five times, then weighed and dried at 50ºC for 48 hrs. The remaining sample was then mixed, sonicated at 70% pulse for 3 minutes, then centrifuged for one hr at 4,000 rpm. The OLF was aspirated off, filtered and rinsed five times with DI water, weighed then dried at 50ºC for 48 hrs. The remaining soil pellet, known as the HF, was then mixed with DI water, centrifuged 5 times, and weighed, dried, then weighed again at 150ºC for 24 hrs after drying.

4.3.3 Total C and N of fractions

Fractions were analyzed for total organic C and N on the Costech ECS 4010 CHNS-O Elemental Analyzer (Valencia, CA, USA) coupled to a Thermo Scientific Delta-V Plus continuous flow isotope ratio mass spectrometer (Waltham, MA, USA) at the UC Merced Stable Isotope Laboratory. The HF from the Merced site was treated further using the acid fumigation method due to effervescence upon the addition of 1 M HCl only in the HF, indicating the presence of inorganic C. The FLF and OLF did not effervesce following the addition of HCl (Harris et al., 2001). Acid-treated HFs were analyzed on the elemental analyzer to determine the concentration of SOC. Soil inorganic C (SIC) was calculated by subtracting the acid-treated C concentrations from total soil C concentrations.

For this method, it is not possible to determine how much C associated to what fraction is lost during the fractionation steps. For the purpose of calculating distribution of recovered C in different fractions (FLF, OLF, HF), we assumed that the recovered mass is equivalent to total mass. Average mass recovery 99.9±1.16%.

4.3.4. Organo-metal complexes in heavy fractions

The HF was used for organo-mineral extractions (Heckman et al., 2018). Briefly, 40 mls of 0.1M sodium pyrophosphate was added to one gram of air-dried ground sample and shaken for 16 hours. Then samples were centrifuged at 20,000 rpm for 20 minutes. Then the extraction was decanted and filtered through a 0.2 µm Whatman GD/X gradient syringe nylon membrane filter into 50 ml falcon tubes. Filtered extracts were then stored at -20 °C until they were ready for analyses.

Extractants were diluted 1:50 with 0.1 M Na-pyrophosphate and analyzed on an Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) for Fe and Al at the UC Davis Analytical Laboratory. Total OC was analyzed on the −20 °C until analyzed for dissolved organic C and N on the total organic C analyzer (TOC-Vcsh, Shimadzu) with a total N module at the UC Davis Analytical Laboratory.

4.3.5. Calcium

The HF from the Merced site was analyzed for extractable Ca^{2+} concentrations using the barium method (buffered to pH 7.0) at the UC Davis Analytical Laboratory (https://anlab.ucdavis.edu/analysis/Soils/430; Rible and Quick, 1960). This site was only selected due to it high levels of inorganic C and strong effervescence only in the HF.

4.3.6. Statistical analyses

The results of this study are presented as treatment means (± standard errors). Shapiro-wilk tests were deployed to determine normality of data for all analyses. A three-way repeated measures analysis of variance (ANOVA) was performed on density fraction samples to determine differences effects between treatment, depth, and site with fraction as a blocking effect. A Tukey's post hoc test was used to determine interactions between these factors. The determination of contributions of total organic C and total N from each fraction was assessed by dividing the C and N by the total for each depth within a site. Error propagation was also taken into account. Treatment effects were analyzed using a two-way ANOVA where treatment and fractions were factors and sites were a blocking effect. A Tukey's post hoc test was used to investigate the interactions between these factors. We used a Pearson's correlation (r) test to determine if %SOC and Ca^{2+} were correlated in the HF at the Merced site and to determine correlations between SOC and Fe and Al. Statistical tests were performed using RStudio 3.3.1.

4-4. Results

4.4.1. Percent distribution of C and N among fractions and depths

Percent distribution of C and N associated to fractions varied depending on fraction, treatment, and depth (Figure 3-1). C and N associated to the FLF was found to be significantly different between depths (C: p=0.02; N: p<0.001) and treatment (C: p=0.02; N: p<0.001) when a three-way repeated measures ANOVA was performed. There was a marginal significance C associated to the FLF between treatment and depth (p=0.06) and between treatment and site (p<0.01) for N associated to the FLF. The FLF accounted for the highest percentage of C and N in the biosolids amended site from 0-10 cm at the Sacramento (C: 85%, N: 82%) and Solano (C:88%; N: 82%). Oppositely, the control (C:58%; N: 54%) in the Merced site had more C and N associated to the FLF than the biosolids treatment (Table 3-1; Table 3-2). Similarly, the C and N associate to the FLF remains higher in the biosolids amended than the control from 30-50 cm (Sacramento: C:47%; N:36%; Solano: C: 47%; N:36%) and 75-100 cm (Sacramento: C:29%; N:28%; Solano: C:27%; N:28%) in Sacramento and Solano. Merced still had more FLF in the control soils from 30-50 cm for C (C:36%) and 75-100 cm (C:13%; N:13%). This indicates that the C and N associated to the FLF is dependent on it's location along the depth of the soil as well as the application biosolids.

Physically protected pools of C and N show that site, treatment, and depth were different along depth, between sites, and treatments. Treatments, depths, and sites were observed to be significantly different (Treatment: both C and N p<0.001; Depth: both C and N p<0.001; Sites: C: p<0.01; N: p=0.03). Interactions were shown between treatment and site (p<0.001) and a marginal significance between treatment, site, and depth (p=0.08). A tukey's post hoc test showed that C associated to the OLF was significantly different between the biosolids and the control from 75-100 cm at the Sacramento site. The control soils had more OLF associated C and N than the biosolids amended at the Sacramento (C:28%; N:35%) and Solano (C:16%) site from 0-10 cm, except for N in the OLF at Solano where the N remained relatively unchanged (Control: 12%; Biosolids; 16%; Table 3-2; Table 3-3). The Merced site had no significant changes for both C and N from 0-10 cm. Similar findings were observed from the 30-50 cm depth where the control soils had more C and N associated to the OLF than the biosolids amended soils, and Merced associated to N remained unchanged but C OLF was higher in the control (33%). In deeper soil, C associated to the OLF was found to be higher in the biosolids amended than the control at both Sacramento and Solano, except for Merced where the differences between the amended and control remain relatively low. Nitrogen in the OLF was higher in the biosolids amended soil only in the Solano site from 75-100 cm. In the Sacramento and Merced site, the control remained higher in N.

The mineral associated fraction followed different trends compared to the OLF and FLF. No treatment effects were observed for both C and N, but site (both p<0.001), depth (both p<0.001) and site:depth interactions were discovered (both p<0.001). From 0-10 cm, the C and N associated to the HF was higher in the control soils in Sacramento (C:53%; N: 26%) and in Solano (C:13%; N:35%). This similar trend is observed in the 30-50 cm and 75-100 cm at Sacramento and Solano where the control soils had more C and N associated to the HF than the biosolids amended. Across all fractions, the Merced site had most of the C and N associated to the HF in the biosolids amended soils down to 100 cm.

4.4.2. Treatment effects of biosolids application on C and N

Treatment effects (biosolids-control) have shown that in many cases (between fractions and depths), there are negative treatment differences, indicating shifts on where SOC is present in the soil depending on site and depth (Figure 4). When assessing the effect size from compiling C content associated to fractions across all sites, we found that overall biosolids application increases C, particularly in deeper soil depths (Figure 3-2a). The FLF had positive effect sizes and has a stronger effect as the C moves down the soil profile. All soil fractions had positive effect sizes from 75-100 cm with the FLF and OLF having the most effect.

All the soil fractions associated to C from all sites experienced positive treatment effects (Biosolids-Control) from 0-10 cm except for the HF at the Merced site (-3.82±2.48% C; Figure 3-4). A two-way ANOVA showed all fractions were significantly different from each other. From the 30-50 cm depth, soil C associated to all fractions increased due to the application of biosolids at the Sacramento site, where treatments and fractions were significantly different from each other (both $p<0.001$; Figure 3-4b). An interaction between treatment and fraction ($p=0.02$) shows that the FLF found in the biosolids amended and control are significantly different from each other ($p=0.01$) from a positive treatment effect of 8.71±0.47%. Both the Solano and Merced site showed a negative treatment effect from the 30-50 cm depth, but in different fractions. Solano had a negative treatment effect of -4.82±4.50% C in the FLF and Merced a negative treatment effect of -4.37±2.58% C in the HF (both sites fractions $p<0.001$). Deep soil depths show positive treatment effects across all sites between all sites, except for the FLF associated C in the Solano site (-3.04±2.98; Figure 3-4c). The Sacramento ($p<0.001$) and Merced ($p=0.04$) site had significant treatment differences between the control and biosolids from 75-100 cm. All sites observed significant differences between fractions (Sacramento: $p<0.001$; Solano $p<0.01$; Merced $p<0.001$). Interactions between treatment and fraction at the Sacramento site from 75-100 cm show that the FLF and the OLF in the control and in the biosolids were statistically different from each other (both $p<0.001$).

Treatment effects on soil total N have shown in some cases negative treatment difference in fractions, indicating loss of total soil N (Figure 3-5). Effect sizes on N follows a similar trend as C where the FLF have increased across all depths, while the effect size of the FLF and OLF remain relatively small and in some cases negative from 0-10 cm and 30-50 cm (Figure 3-2b). Deep soil shows all fractions have positive or zero effect size, similar to C. From 0-10 cm positive treatment effects were observed at the Sacramento and Solano sites across all fractions, except for the Merced site (Figure 3-5). Significant treatment differences were observed at the Sacramento site ($p<0.01$) when a two-way ANOVA was performed. All sites experienced significant differences between fractions (all $p<0.001$) and Solano and Merced had treatment and site interactions (both $p=0.04$). The N associated to the FLF and the OLF at the Sacramento site experienced positive treatment effects, except for the HF where it remained relatively unchanged from 30-50 cm (Figure 3-5b). Significant differences were observed between treatment ($p<0.001$), fraction ($p<0.001$). An interaction between treatment and fraction ($p<0.01$) showed differences between the N associated to the FLF and OLF between control and biosolids (both $p<0.001$). All the fractions at the Sacramento site experienced positive treatment effects from the 75-100 cm depths (Figure 3-5c). Significant differences were observed between treatments ($p<0.001$), fractions ($p<0.001$), and interactions ($p<0.01$). The Solano site experienced a negative treatment effect for N associated to the FLF from 75-100 cm (fraction $p<0.01$). The Merced site had a slight negative treatment effect in the N associated to the HF from 75-100 cm (-0.08±0.10% N), but soils amended with biosolids were statistically different form the controls ($p=0.04$), and fractions were also significantly different from each other ($p<0.001$).

4.4.3. Correlations of organo-mineral complexes

At the Sacramento site, the control soils showed that TOC was positively associated along depths to Fe and Al, though not significant (all $p>0.05$; Table 3-4). Only from 75-100 cm was there a negative association between Fe and TOC ($r=-0.397$, $p=0.74$). Biosolids amended soils at the Sacramento site show stronger correlations between TOC to Fe and Al from 0-10 cm and 30-50 cm though not significant except for Al from 30-50 cm ($p=0.04$). From the 75-100 cm depth negative correlations were observed for both Fe and Al associated to TOC in the biosolids amended soils (both $p>0.05$, Fe: $r= -0.77$; Al: $r= -0.40$). Control soils at the Solano site show moderate correlations between TOC and Fe from 0-10 cm ($r=0.41$, $p=0.73$) and from 75-100 cm ($r=0.228$, $p=0.924$), with weaker correlations observed from the 30-50 cm depth ($r=0.02$, $p=0.98$). Correlations between TOC and Al show in the Solano control soils show moderately negative correlation from 0-10 cm ($r= -0.70$, $p=0.52$) and an increasing positive correlation along depth (30-50 cm: $r=0.05$; 75-100 cm: $r=0.397$; both $p>0.05$). Biosolids amended soils at the Solano site show that at TOC and Fe are weakly correlated from 0-10 cm ($r=0.1$), negatively correlated from 30-50 cm ($r= -0.996$; $p=0.052$), and weakly correlated again from 75-100 cm ($r=0.228$, $p=0.92$). The TOC and Al correlations in the biosolids amended soils at Solano also had negative correlations from the 0-10 cm ($r=-0.05$, $p=0.96$) and from 30-50 cm with a significant negative Pearson's correlation ($r=-1$, $p<0.001$). Deep soils that were amended with biosolids had a weak correlation between TOC and Al ($r=0.30$, $p=0.81$).The Merced site had weak correlations between TOC to Fe and Al for both treatments along depths, except for the correlation between TOC and Al in the biosolids amended soil from 0-10 cm ($r=0.99$, $p=0.03$). Some concentrations of Fe and Al were undetectable and Pearson correlations could not be performed (Controls: TOC:Al from 0-10 cm; TOC:Al from 30-50 cm; TOC:Fe/Al from 75-100 cm; Biosolids: TOC:Al from 30-50 cm; TOC:Fe from 75-100 cm).

4.4.4. Correlations between soil organic C and Ca^{2+} in Merced

The HF from the Merced site, stronger correlations between Ca^{2+} and SOC% were observed from the control soils than the long term amended soils across all depths and sites (Figure 6). Correlations were strongest at the 0-10 cm for each treatment compared to other depths where the control had an correlation coefficient of 0.94 ($p=0.22$) and the biosolids amended had an r of 0.03 ($p=0.98$). The 30-50 cm showed a slight negative correlation in the amended soils ($R^2=-0.09$, $p=0.93$), but positive correlations in the control soils ($r=0.47$, p=0.70). Finally, the 75-100 cm depth showed strong negative correlations in the biosolids soil with a r of -0.88 (p=0.31) and the control showed minimal correlation between Ca^{2+} and SOC% though not negative (r=0.31, p=0.80).

4-5. Discussion

4.5.1. Effects of long-term biosolids applications on soil density fractionations

Fractionation techniques are a valuable tool that can provide indications on the fate of the C and N from organic matter amendments and the long-term C storage potential of a soil (Grunwald et al., 2017; Ryals et al., 2014). In our study, we found that relative proportions of C and N found in each fraction differed along depth, sites, and treatment. Effect sizes across our study shows the impact of how organic amendment application, like biosolids, and measuring deep soil C can provide a better assessment on the C sequestration potential of a soil. The effect size of both C and N, the FLF increased in surface soils, and strengthens from 30-50 cm even though biosolids are only incorporated to 30 cm. Across all fractions, more pronounced treatment effects are found in deeper soils, where all fractions had higher SOC pools. As SOC and total N moves to subsoils, the turnover rate tends to decrease; thus, remaining for longer periods of time (Tautges et al., 2019). Subsoils also typically contain higher concentrations of clays and can promote formation of effective organo-mineral complexes that can contribute to C stabilization (Kögel-Knabner et al., 2008).

As expected, total organic C and N content associated with the FLF from 0-10 cm and 30-50 cm increased in the biosolids amended soil, but only in the Sacramento and Solano site. The higher proportions of FLF in the amended soils from Sacramento and Solano site are likely from two sources. The first likely source is the C and N from the biosolids that persisted over time. A 547 day incubation study by Baldock et al. (2021) assessed the chemical composition and decay rates of organic amendments like biosolids, (Baldock et al., 2021) and found that biosolids tended to emit less CO_2 than other composts and that most of the C from biosolids was allocated in slow mineralizing pools in soil. The second likely source of additional FLF C and N is from above- and belowground plant litter as a result of biosolids promoting higher plant productivity (Ryals and Silver, 2013). Some studies have found that biosolids have increased SOC and plant productivity after a one-time application (Brown et al., 2011; Wijesekara et al., 2017). Grasses are known to have deep roots that can reach down to deep soil depths; here, C and N may also be contributed through root turnover (Silver et al., 2010). One study found similar results on the distribution of C following the application of biosolids, particularly in the biologically active layer of the soil (0-10 cm; Silva et al., 2015). In addition, they found that there was active vertical transport into deeper soil layers showing increased input from plant roots (Silva et al., 2015).

Deeper soil depths show higher proportions of C and N attributed to the FLF and OLF only at the Sacramento and Solano site. Control soils showed that the C and N present at the Sacramento and Solano sites had organic C and total N associated to HF. Similarly, the N associated to the HF were higher in the control soils than the amended soil. The higher proportions of C and N associated to HF in the control soils may be attributed to less accumulation of FLF C directly from biosolids application and the promotion of plant production (Brown et al., 2011; Silva et al., 2015).

The Merced site did not experience an increase in C and N associated to FLF from 0-10 cm or from 30-50 cm, similarly N did not increase from 0-10 cm, but the 30-50 cm remained unchanged. When comparing the unamended control and the biosolids amended soils at Merced, a shift in C and N associated to fractions differ. Unamended controls had higher proportions of C and N associated to the FLF than the amended soil where higher proportions of C and N was associated to the HF (Table 2 & 3). This shift from FLF to HF indicates that C and N from 0-10 cm and 30-50 cm in amended soils is changing chemically and binding to mineral surfaces instead of "free-floating" as observed in the control (Duddigan et al., 2019). Carbon inputs from biosolids and increasing plant production have been shown to can enhance soil microbial activity (Fernandes et al., 2005; Hamdi et al., 2019). This produces microbially-derived compounds that are the primary constituents of stable SOM which can further promote the formation of long-term SOM storage (Marín-Spiotta et al., 2008; Poeplau et al., 2018). Soil management may also influence this more C and N associated to HF through practices such as flood irrigation and tillage. The Merced site has coarse texture, has had the fewest frequency of biosolids application, soils are used for an alfalfa-corn rotation resulting in tillage, and is flood irrigated. Tillage breaks up soil structure and reduces aggregates, resulting in C loss in surface soils from erosion or through mineralization from microbes that have access to C that were previously protected in aggregates (Stewart et al., 2012).

4.5.2. Organo-mineral complexes is dependent on pH

Complexation of SOC to Al and Fe was found to be more strongly correlated at the Sacramento and Solano sites than the Merced site (Figure . We computed the C:M ratio (Fe+Al mols) to determine whether there is enough stock of metal ions in the soil for chelation to have significant influence on C storage in soils across the three sites, treatments, and along depth. Following Oades (1989), Masiello et al. (2004), and Berhe et al. (2012), we assumed each organic C functional group represents six C atoms associated with one negative charge. If every OC functional group was bound to a metal ion and all metal ions existed in the +1 charge state, the molar C:M ratio would be six (Masiello et al., 2004). Any ratio above 6 would indicate that there is not enough stock for Al and Fe to bind to SOC. However, it can be assumed that not all SOC and metal ions will fall within these assumptions. Some OC can have many functional groups that can interact with one metal ion, and some metal ions exists as polycations (raising the C:M ratio; (Berhe et al., 2012; Masiello et al., 2004). Hence, bonding of all OC to M seems to fall within a ratio range between 2 to 10 (Oades, 1989).

At Sacramento and Solano, the C:M ratio generally falls within the 2 to 10 range (Figure 3-6), except in the biosolids amended soil at the Solano site from 30-50 cm and 75-100 cm. This may be due to more C introduced into the soil from the biosolids application causing the C:M ratio to increase and fall out of this range. Thus, indicating there is not enough Fe and Al ions to bind to the excess C present in the soil. The Merced site had a very large C:M ratio ranging from 100-350. One possible explanation for this findings is that the Merced site has a pH of 8.0, the solution used for this procedure, sodium pyrophosphate, acts as a chelating agent and is used to estimate the amount of metal ions involved in soil via alkali-induced organic matter dissolution (Coward et al., 2018, 2017). Both the Sacramento and Solano site had a pH of 5.9 in the top 30 cm of soil making these sites more ideal candidate for estimating organo-mineral complexes. The alkalinity of the soil at the Merced site could potentially interfere with the extraction procedure since acidic pH drives the sorption of organic matter onto metal nanoparticles, hence, potentially causing inconsistent results (Coward et al., 2017).

Biosolids have been shown to increase Fe and Al in soils due to the direct contribution of these metal ions to soil. One long-term study found that thirty years of biosolids application significantly increased amorphous Fe and Al in mined soils (Tian et al., 2013). They also found increases in mineral-associated organic matter in biosolids amended soils, indicating that the C and N in these plots remain for longer periods of time. Mikutta et al. (2006) found positive correlations between mineral associated organic matter and soil amorphous Fe and Al. Biosolids contain high Fe and Al in amorphous form, thus introducing more metal ions in the soil. (Mikutta et al., 2007; Tian et al., 2013). At certain conditions, the introduction of metal oxides found in biosolids and SOC can become intimately related due to organic matter carrying negative charges and metals having positive ones (Chiu and Tian, 2011). Organic-metal associations primarily occurs for the amorphous Fe and Al oxides because of their large surface area (Kleber et al., 2005). The introduction of biosolids only facilitates the association of metal oxides to SOC which results in higher organo-mineral associations (Chiu and Tian, 2011). At the Sacramento site, the biosolids amended soils had a lower C:M ratio than the control indicating that biosolids application increased the total amount of Fe and Al bound to SOC. In the Solano site, the biosolids amended soils had a higher C:M ratio, indicating that there is more C being introduced than is present in the biosolids or in the soil. Since the composition of the biosolids applied were not statistically different across sites, it could be deduced that due to the yearly, high frequency of biosolids application, there was more Fe and Al present in these soils compared to the Solano and Merced site.

4.5.3. Biosolids application influences C associations to Ca^{2+} in the Merced site

Mechanisms that influence how Ca^{2+} interacts with SOC are influenced by high pH in soils where positively charged ions are increasingly adsorbed to surfaces with variable charges such as clays, iron and aluminum oxides, and SOM . At the Merced site, soils had a pH of 8 from 0-100 cm and a high percentage of soil inorganic carbon (SIC; ~22% of total C) in the form of calcium carbonate ($CaCO_3$) (Villa and Ryals, 2021). Studies in unmanaged soils have found strong correlations between Ca^{2+} and SOC content and stabilization (Martí-Roura et al., 2019; Rowley et al., 2021, 2018; Solly et al., 2020). Hence, we considered the role Ca^{2+} may play in C sequestration at the Merced site.

We hypothesized that the increasing SOC content from biosolids application would be correlated with Ca^{2+}; hence, indicating that the Ca^{2+} may be associated to SOC chemically or physically. However, when comparing the correlations between the controls and the amended soils, we found the opposite to be true (Figure 7). In fact, there is an increasing negative correlation moving down the soil profile in the amended soil compared to the control, where moving down in depth just shows a weaker positive correlation. Soil organic matter has a high abundance of negatively charged sites which can improve cation exchange capacity, thus increasing soil C content in soils by reacting as a surface area in soil where SOC may be adsorbed (Solly et al., 2020). Some studies have found that the application of organic matter amendments increase SOC and CEC (Diacono and Montemurro, 2010). However, CEC is strongly associated to the available surface area of the soil (Farrar and Coleman, 1967; Solly et al., 2020), effectively influencing the ability for SOC to adsorb to reactive soil surfaces (Solly et al., 2020). One study found that there was no significant increase of CEC in a coarse textured almond orchard after application of green waste compost and composted wood-chip manure (Villa et al., 2021). The Merced site has a uniform soil texture of sandy loam from 0-100 cm, the percentage of sand ranged from 53% (0-10 cm)-73% (75-100 cm; Villa and Ryals, 2021).

The chemical composition of the biosolids could also contribute to the negative correlation with Ca^{2+}. Biosolids are rich in Fe, and the biosolids applied at all three these sites were similar in composition, and had a concentration of 35±19 g/kg (Table 3-2; Villa and Ryals, 2021). It is possible that the Fe in the biosolids interacts with the C that is already present in the biosolids indicating preferential binding. The strength of metal cations in binding organic compounds largely depends on the size of the hydration shell and the valence of electrons, Fe has a 3+ charge; therefore, a larger hydration shell than Ca^{2+} (Solly et al., 2020).

4-6. Conclusions

Our study assesses the mechanisms that influence C sequestration in three ecosystems that have had biosolids application for twenty-years. Density fractionations showed that amended soils had more C and N associated to the microbially accessible, "free-floating" fraction down to 100 cm depths in the Sacramento and Solano site. This was likely to the promotion of plant production and vertical movement of C and N to deeper soil depths. The Merced site had more C and N associated in HF in amended soils relative to the control suggesting the chemical composition of the C and N from biosolids application is changing. Organo-complexes were shown to be a stabilization mechanism in the Sacramento and Solano site indicating that the C associated to these metal ions will remain stabilized for long periods of time. Negative correlations are shown between SOC and Ca^{2+} along depth relative to the control, implying that the biosolids themselves may be interacting with the C from the biosolids and soil. Our study indicates that biosolids increase SOC not only in shallow soils, but also deep soils where it can stay for long periods of time across different agroecological contexts.

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4.8. Tables

Table 4-1 Detailed soil characteristics from each site. Values are site means ± standard error.

Site	Depth	pH of Biosolids Amended Soils	pH of Control Soils	% Sand	%Silt	%Clay	Texture
Sacramento	0-10 cm	6±0	6±0	35±2	45±1	20±4	Clay Loam
	10-30 cm	6±0	7±0	35±4	40±3	24±1	Clay Loam
	30-50 cm	7±0	7±0	35±4	40±3	24±1	Clay Loam
	50-75 cm	7±0	7±0	44±11	33±5	23±2	Clay Loam
	75-100cm	7±0	7±0	47±8	30±6	15±6	Clay Loam
Solano	0-10 cm	6±0	6±0	48±5	30±3	22±3	Clay Loam
	10-30 cm	6±0	6±0	45±4	27±3	28±3	Clay Loam
	30-50 cm	7±0	7±0	46±5	22±3	32±3	Clay Loam
	50-75 cm	8±0	7±0	43±4	24±4	33±1	Clay Loam
	75-100 cm	9±0	8±0	50±9	27±4	23±7	Sandy Clay Loam
Merced	0-10 cm	8±0	8±0	53±8	33±5	14±3	Sandy Loam
	10-30 cm	8±0	8±0	58±5	31±3	12±2	Sandy Loam
	30-50 cm	8±0	8±0	68±3	23±2	10±1	Sandy Loam
	50-75 cm	8±1	8±1	75±3	19±3	6±1	Sandy Loam
	75-100 cm	8±0	8±0	73±1	20±1	7±1	Sandy Loam

Table 4-2: The chemical composition of C, N, C:N, and Fe of biosolids from ten wastewater treatment plants. Values were averaged, standard deviations are shown. Values in brackets are the minimum and maximum value. One-way ANOVA was used to determined significant differences as described in Villa and Ryals, 2021. The biosolids composition of the elements below were not statistically different from each other across the wastewater treatment plants ($p<0.05$).

Nutrients	**Biosolids Composition**
C (mg/kg)	381.2±32.6 (338-443)
N (mg/kg)	58.3±9.5 (49.2- 74.7)
C/N	6:1 (5:1-7:1)
Fe (mg/kg)	34.8±19.2 (7.3- 64)

Table 4-3: Proportions of SOC recovered in FLF, OLF, and HF between treatments along depths.

Site	**Treatment**	**Carbon Depth**	**FLF%**	**OLF%**	**HF%**
Sacramento	Biosolids	0-10	85%	12%	3%
	Control		19%	28%	53%
Solano	Biosolids	0-10	88%	2%	10%
	Control		71%	16%	13%
Merced	Biosolids	0-10	12%	28%	60%
	Control		58%	21%	21%
Sacramento	Biosolids	30-50	47%	36%	17%
	Control		19%	28%	53%
Solano	Biosolids	30-50	88%	2%	10%
	Control		27%	14%	59%
Merced	Biosolids	30-50	11%	6%	83%
	Control		34%	33%	32%
Sacramento	Biosolids	75-100	29%	34%	37%
	Control		4%	28%	68%
Solano	Biosolids	75-100	27%	55%	17%
	Control		15%	21%	64%
Merced	Biosolids	75-100	5%	3%	92%
	Control		13%	8%	80%

Table 4-4: Proportions of N recovered in FLF, OLF, and HF between treatments along depths.

Site	Treatment	Nitrogen Depth	FLF%	OLF%	HF%
Sacramento	Biosolids	0-10	82%	12%	5%
	Control		39%	35%	26%
Solano	Biosolids	0-10	71%	16%	13%
	Control		53%	12%	35%
Merced	Biosolids	0-10	12%	26%	62%
	Control		54%	26%	20%
Sacramento	Biosolids	30-50	36%	30%	34%
	Control		10%	14%	76%
Solano	Biosolids	30-50	88%	2%	14%
	Control		17%	7%	75%
Merced	Biosolids	30-50	18%	12%	69%
	Control		18%	12%	69%
Sacramento	Biosolids	75-100	30%	19%	51%
	Control		2%	25%	73%
Solano	Biosolids	75-100	28%	41%	31%
	Control	75-100	9%	10%	81%
Merced	Biosolids	75-100	10%	8%	81%
	Control		13%	11%	75%

Table 4-5: Concentrations of TOC, Fe and Al from pyrophosphate extractions (Fe$_{py}$ and Al$_{py}$). Ratios of TOC: Fe$_{py}$ and TOC: Al$_{py}$ and Pearson correlations are shown. NA values are due to undetectable concentrations of Fe or Al. Astericks represent significance ($p<0.05$*; $p<0.01$**; $p<0.001$***).

Site	**Treatment**	**Depth (cm)**	**Al$_{py}$ (mg/g)**	**Fe$_{py}$ (mg/g)**	**TOC (mg/g)**	**TOC:** Fe$_{py}$	**Pearson Correlations**	**TOC:** Al$_{py}$	**Pearson Correlations**
Sacramento	Biosolids	0-10	0.48±0.00	1.25±0.00	1.47±0.19	1.17	r=0.95	3.08	r=0.88
		30-50	0.84±0.00	0.57±0.00	0.65±0.17	1.14	r=0.98	0.77	r=0.99*
		75-100	0.41±0.02	0.27±0.00	0.62±0.17	2.31	r=-0.84	1.50	r=-0.8
	Control	0-10	0.91±0.00	0.82±0.00	2.03±0.02	2.47	r=0.88	2.23	r=0.99
		30-50	0.64±0.00	0.37±0.00	0.96±0.13	2.59	r=0.629*	1.49	r=0.99*
		75-100	0.64±0.00	0.46±0.00	0.25±0.04	0.55	r=0.39	0.40	r=0.39
Solano	Biosolids	0-10	0.44±0.19	0.54±0.20	2.05±0.13	3.78	r=-0.11	4.69	r=-0.05
		30-50	0.22±0.20	0.21±0.15	1.73±0.10	8.38	r=-0.99*	7.83	r=-1.00***
		75-100	0.1±0.14	0.07±0.10	0.730.10	9.88	r=0.761	7.42	r=0.23
	Control	0-10	0.31±0.32	0.36±0.29	2.15±1.73	5.97	r=0.41	7.00	r=-0.69
		30-50	0.26±0.44	0.19±0.28	0.85±0.27	4.56	r=0.02	3.29	r=0.05
		75-100	0.22±0.05	0.03±0.06	0.61±0.04	18.28	r=0.23	2.77	r=0.40
Merced	Biosolids	0-10	0.01±0.14	0.03±0.12	1.8±0.22	53.79	r=0.45	217.41	r=0.359
		30-50	0.00±0.04	0.01±0.02	0.86±0.08	63.84	r=0.62	NA	NA
		75-100	0.02±0.03	0.01±0.02	0.55±0.17	50.52	NA	30.74	r=0.94
	Control	0-10	0.01±0.01	0.03±0.02	1.93±0.15	56.89	NA	177.60	NA
		30-50	0.00±0.09	0.01±0.06	0.82±0.18	56.50	r=-0.78	593.95	NA
		75-100	0.00±0.04	0.01±0.00	0.41±0.16	42.46	NA	149.55	NA

4.9. Figures

Figure 4-1). Average percent C from 0-10 cm, 30-50 cm, and 75-100 cm across all sites. Error bars show standard error (n=3).

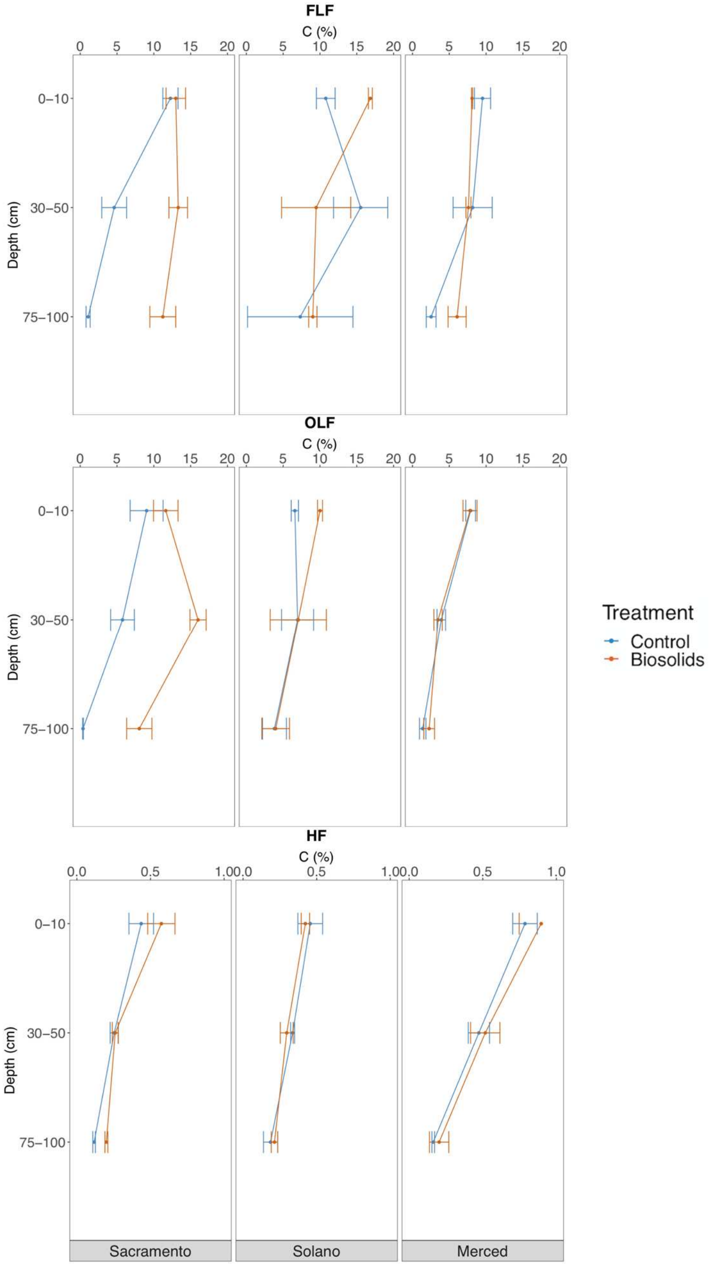
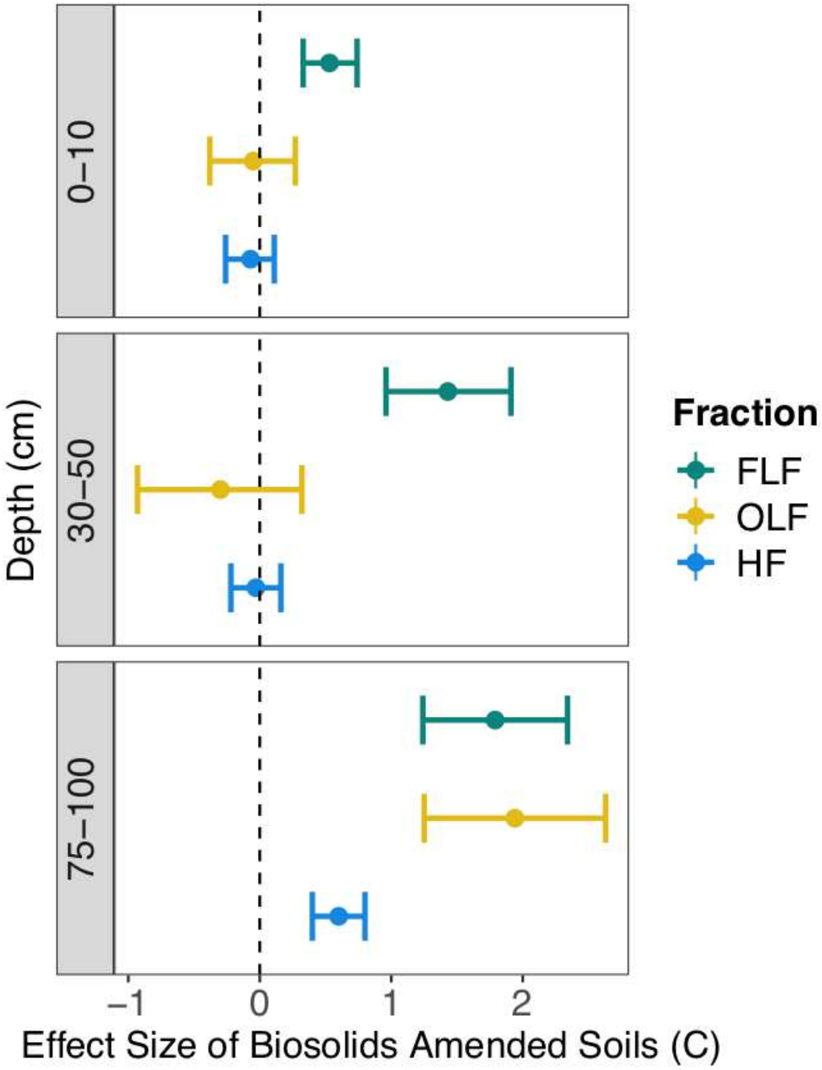


Figure 4-2 a-b) Plot showing effect size of a) C% and b)N% of different fractions across all sites. Error bars are showing the confidence interval (95%).

a)



b)

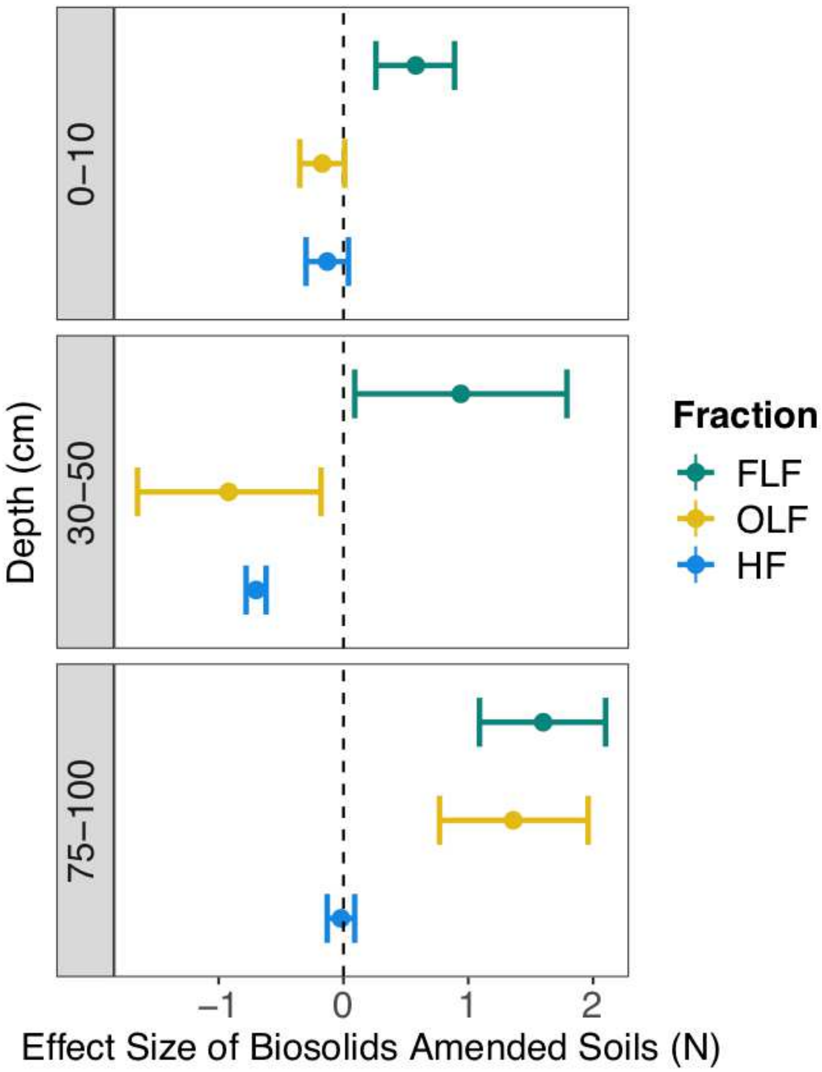

0–10
30–50
75–100
Depth (cm)
Fraction
FLF
OLF
HF
−1
0
1
2
Effect Size of Biosolids Amended Soils (N)

Figure 4-3 a-c). Average percent N in fractions from 0-10 cm, 30-50 cm, and 75-100 cm across all sites. Error bars show standard error (n=3).

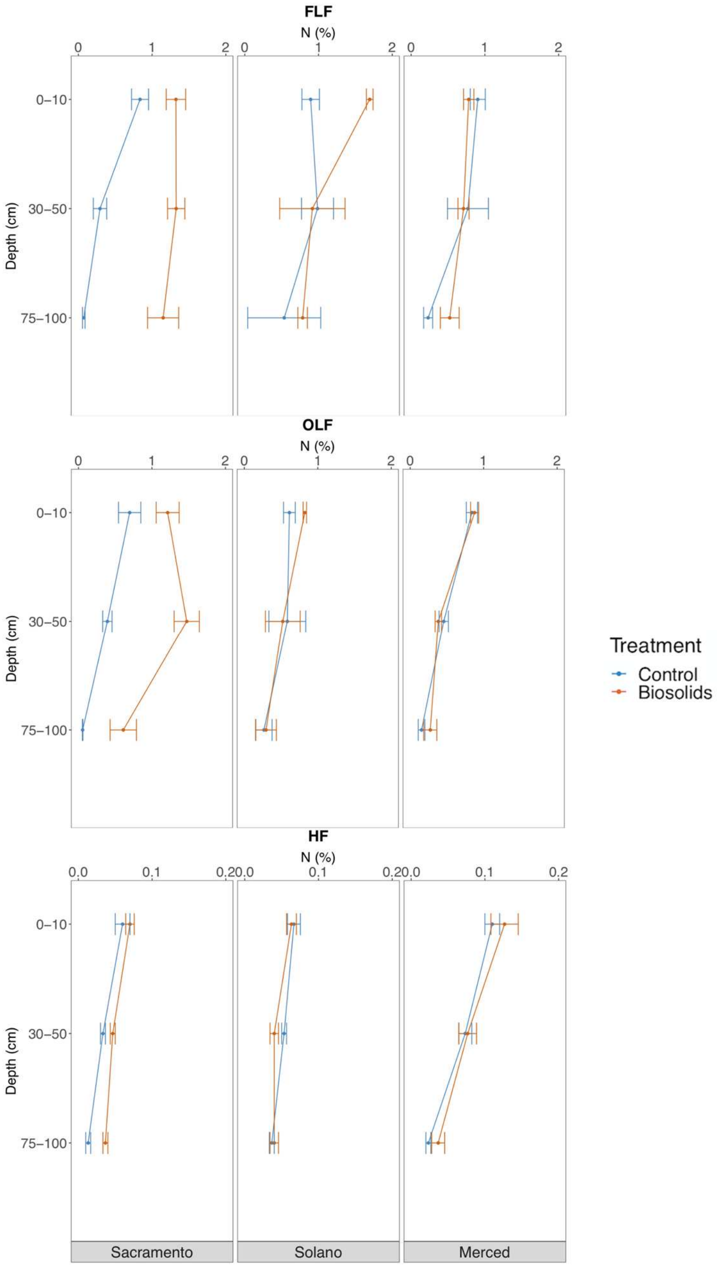


Figure 4-4a-c.) The treatment effect of biosolids application on percent C in each soil fraction from the three depths: from a) 0-10 cm, b) 30-50 cm, and c) 75-100 cm. Error bars are standard errors.

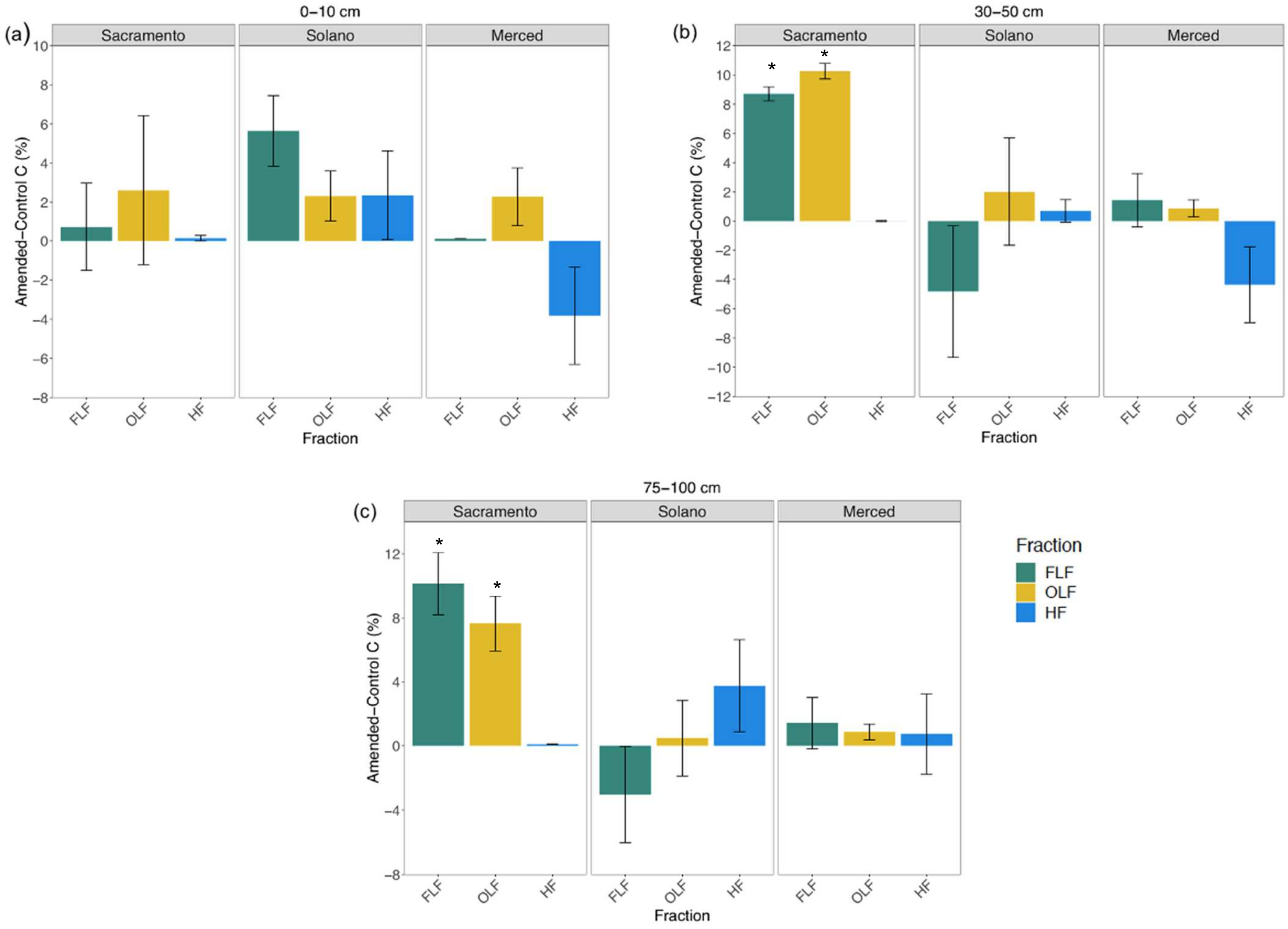


Figure 4-5a-c). The treatment effect biosolids application on percent N in each soil fraction from the three depths: from a) 0-10 cm, b) 30-50 cm, and c) 75-100 cm. Error bars are standard errors.

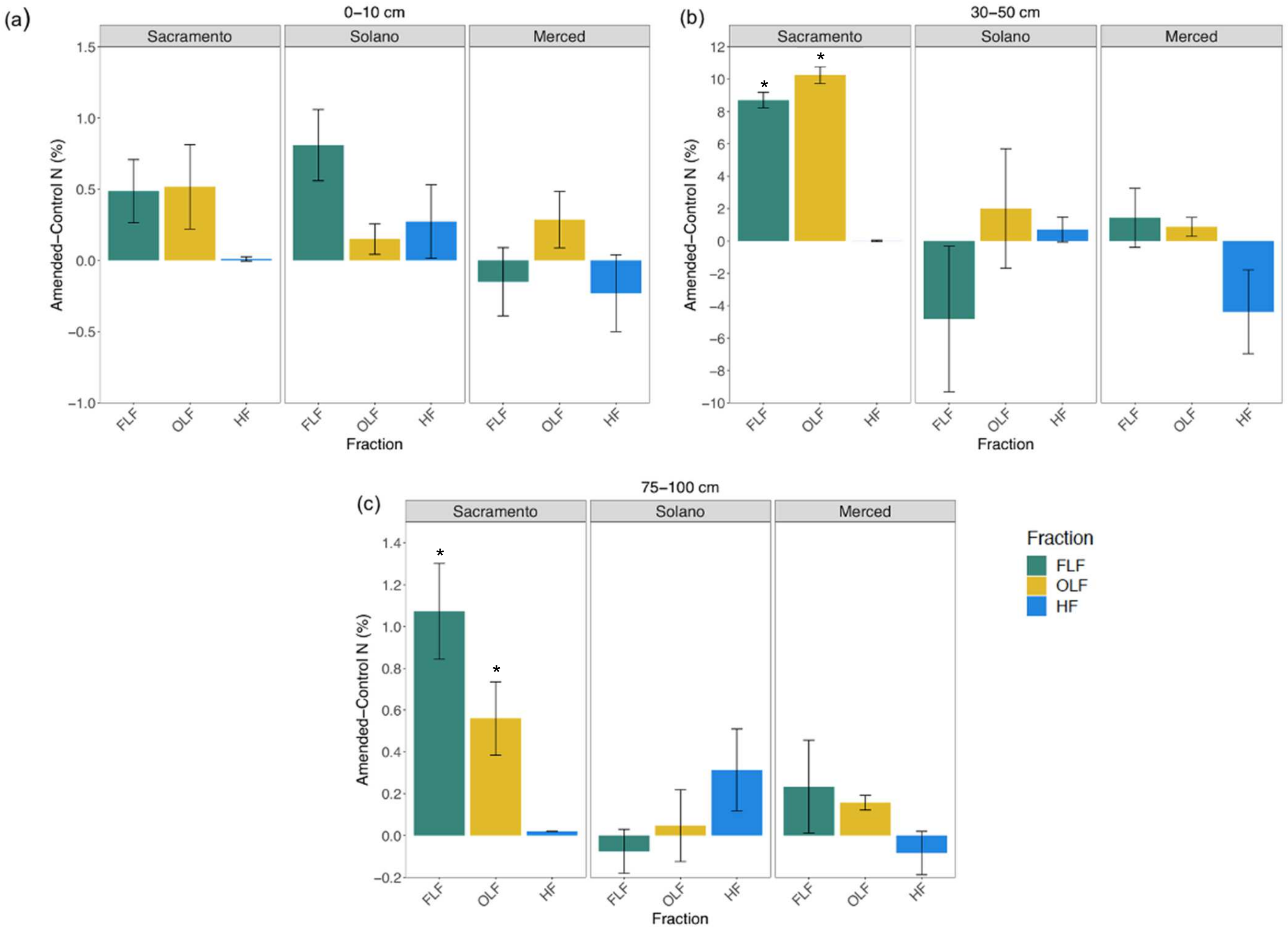


Figure 4-6: Ratio showing moles of C per moles of M (Fe + Al) along soil depth across three sites.

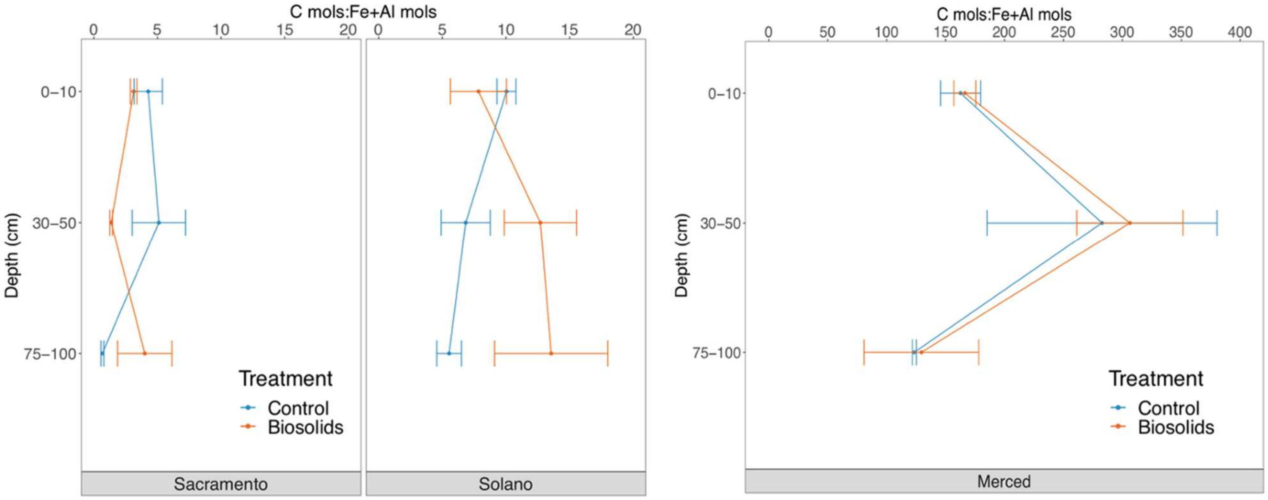
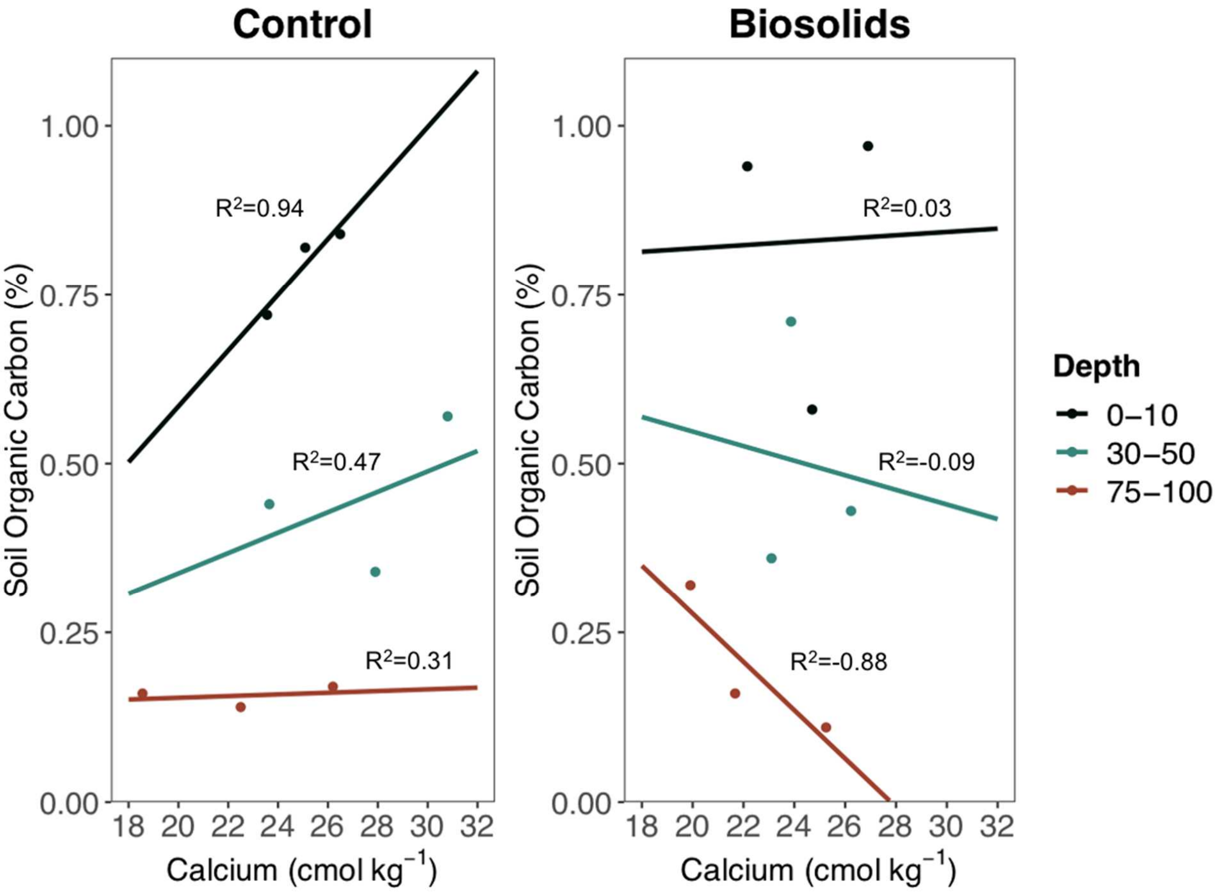


Figure 4-7: Relationship between SOC% and Ca^{2+} in the biosolids amended and control at the Merced site using a Pearson correlation test.



5 Conclusion

Understanding how sustainable management practices influence soil fertility and carbon sequestration can have important implications on the resilience of soils across California's diverse agroecological contexts. This dissertation spans a broad spectrum of agricultural practices, site conditions, time, and organic matter amendment types. These studies range from fertilized almond orchards amended with two composts types (composted manure, composted greenwaste) on different soil textures (Chapter 1); a biosolids application study in three agricultural sites where management and application frequency varied during a twenty-year period (Chapter 2); finally, a study that focuses on the mechanisms of carbon persistence in these soils that have had long-term amended biosolids applied across these three sites (Chapter 3).

I found that readily available composed manure and composted green waste improved soil fertility in two almond orchards with contrasting soil textures in only two years. The type of organic matter amendment did not influence soil nutrient cycling differently across both orchards (Loam and Sand); however, soil texture did play a dominant role. This demonstrated that the potential benefit of using these organic amendments can vary depending on site conditions, and growers should take these conditions into consideration in order to optimize benefits of organic matter amendments. These findings are consistent in the long-term biosolids amended study (Chapter 2 & 3). Two sites with fine textured soils (Sacramento and Solano) resulted in a higher relative change in carbon content compared to the site with a coarse soil texture (Merced).

This dissertation shows how organic amendments contribute to increases in carbon concentrations and result in co-benefits for soil health (e.g. increased soil microbial biomass carbon and nitrogen, increased soil nitrogen concentrations). However, these effects vary across management practices and soil conditions. Findings from Chapter 2 and 3 show that despite having the highest frequency of biosolids applied, significant increases in carbon were restricted to surface soils (0-10 cm). Carbon found at this site was associated with less persistent forms of carbon and is expected to cycle through the soil system relatively quickly. Sacramento soils were flood irrigated which may explain how carbon and nitrogen can potentially be lost from the soil system. Organo-metal associations are heavily mediated by pH, both the Sacramento and Solano site had a pH of 5.9, and the association of carbon to metals such as iron and aluminum were high. This suggests that the carbon that is associated to metals is strongly bound and remains in the soil for long periods of time (Coward et al., 2017). The Solano site had the least amount of biosolids applied, yet the highest relative change in terms of carbon content down to 100 cm depths. This site was a rainfed grassland managed for grazing. Increased carbon at this site occurred in all three soil fractions (the free-light, occluded-light, and the heavy fraction) down to deep soil layers. This finding indicates that application rate does not determine C sequestration potential . Finally, the Merced site had moderate amounts of biosolids applied, but no significant increases in soil carbon or nitrogen content was observed. This site showed that management (flood irrigation and tillage) and soil conditions (high pH of 8.0) may influence the ability for carbon to increase and remain in the soil. Increases in carbon at the Merced site was associated to the heavy fraction of the soil, and the most relative increase was found from the 75-100 cm depth. Correlations between calcium and carbon were found to become weaker when biosolids were introduced. Showing that the composition of the biosolids are interacting with carbon showing that there could be preferential binding to the biosolids instead of calcium in the soil.

The reuse of wasted organics has significant implications on closing nutrient loops through an ecological approach to soil nutrient management and has the potential to contribute to sustainable management goals (Ryals et al., 2021). My dissertation research has shown that recycling waste streams to agricultural soils can provide plant available nutrients to support plant production as well as organic matter to improve soil health and ecosystem services. These findings indicate that there are both short-term and long-term benefits from ecological approaches to properly manage nutrients, reduce waste, and foster more resilient agricultural soils.

Overall, my dissertation shows the importance of improving soil management through the recycling of organic amendments in agriculture. Agricultural soils contribute to greenhouse gas emissions through poor management, tillage, fertilizer usage, livestock, and conversion of natural ecosystems to agriculture (Paustian et al., 2016). In addition, soils have an ability to store large amounts of carbon, which make it susceptible to poor management changes causing them become either a source to emissions, further contributing to climate change. The results of my dissertation shows that improving soil fertility and increasing carbon sequestration is possible by using composts and biosolids to agricultural soils. These findings should be useful for the development and implementation of California policies that prioritize soil carbon sequestration, specifically in agricultural or grasslands systems, such as the Healthy Soils Project and the Senate Bill 1383. The relevance of this work can be also be useful at a global scale; specifically the implementation of nature-based climate solutions, such as the Paris 4 per mil or the 30x30 plan.

5.1 References

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