Title

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Journal

Journal of Environmental Quality, 50(5)

ISSN

0047-2425

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Publication Date

2021-09-01

DOI

10.1002/jeq2.20270

Peer reviewed

Received: 6 April 2021 | Accepted: 19 July 2021

DOI: 10.1002/jeq2.20270

Journal of Environmental Quality

TECHNICAL REPORTS

Resource Recovery and Reuse

Soil carbon response to long-term biosolids application

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Assigned to Associate Editor Martin Tsui.

Abstract

Astudy was conducted in three agroecosystems in California (Sacramento, Solano, and Merced counties) that received biosolids applications for 20 yr. 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$_{dry}$ ha^{-1}, resulting in the addition of 26 (Solano), 36 (Merced), and 125 (Sacramento) Mg biosolids-C ha^{-1}. Measurements included soil organic carbon (SOC) and total nitrogen (N) concentrations from 0 to 100 cm and microbial biomass C (MBC) and microbial biomass N (MBN) from 0 to 30 cm in biosolids-amended and control sites. Biosolids treatments had greater amounts of SOC and total N at all sites, and MBC and MBN were greatest at Sacramento and Solano. The 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 ($p < .001$). 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 suggest 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.

1 | 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 (P), plant-available nitrogen (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) and promote biological soil processes, such as nutrient mineralization and soil respiration (Carbonell et al., 2009; Garcıá-Gil et al., 2004; Lloret et al., 2016).

Land application of biosolids has 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 (*Triticum* L.) production

Abbreviations: MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; SIC, soil inorganic carbon; SOC, soil organic carbon; UC, University of California 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), whereas 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 biosolids-amended soils is variable due to climatic conditions, management, and properties of the soil and biosolids (Wijesekara et al., 2017). Therefore, more localized, comparative, and mechanistic field research is needed to assess the potential to use biosolids as a means to increase soil C.

Although 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 & Don, 2015; Tautges et al., 2019; Yost & Hartemink, 2020). Shallow sampling leads to an incomplete understanding of the effect of biosolids land application on soil C storage and can result in an underestimation of a soil's potential to sequester C (Lorenz & 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 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 best use of this underutilized organic resource. The purpose of this study was to determine the effects of long-term (20 yr) 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 hypothesized that SOC will increase below the depth of biosolids incorporation (~30 cm).

Core Ideas

- Deep soil accounting is needed to better estimate a soil's climate change mitigation potential.
- C sequestration is dependent on environmental factors and management practices.
- Application frequency and amount were found to not correlate with increases in C concentrations.

2 | MATERIALS AND METHODS

2.1 | 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′38.0 W), 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 Natural Resources Conservation Service 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, 10–30, 30–50, 50–75, and 75–100 cm) at every 10 m 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 University of California (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 d, 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 yr corn (*Zea mays* L.) and 4 yr alfalfa (*Medicago sativa* L.) 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, mean annual precipitation, mean annual temperature, and biosolids application rates at each site are shown in Supplemental Table S1.

TABLE 1 Site characteristics and biosolids application information

Site	Transect no.	Frequency of application	Total amount applied from T = 0	Estimated C applied	N applied	pH (0–30 cm)	Soil texture	MAT	MAP
			Mg dry biosolids ha^{-1}	——Mg ha^{-1}——				˚C	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 yr	82	29	4	5.9 ± 0.2	clay loam	23	630
	5	every 3 yr	86	30	5				
	6	every 3 yr	53	18	3				
Merced	7	every 5 yr	157	54	8	8.0 ± 0.1	sandy loam	18	250
	8	every 5 yr	65	23	4				
	9	every 5 yr	93	32	5				

Note. MAP, mean annual precipitation; MAT, mean annual temperature

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-yr timeframe (Sacramento: yearly 1999–2018; Solano: every 3 yr 2005–2017; Merced: every 5 yr 1995–2011). Biosolids were surface applied and incorporated via tillage to a soil depth of ∼30 cm. Controls in the Sacramento and Solano sites that did not receive biosolids application were not tilled; however, Merced was tilled because alfalfa and corn were still grown on the control soil. Application of biosolids is prohibited within a predetermined 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.

2.2 | 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-yr 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 N. 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 10 wastewater treatment plants that provide biosolids to farmers in the study region. 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, personal communication, 2021). 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, personal communication, 2021). 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). 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 by inductively coupled plasma atomic emission spectrometry for total P, potassium (K), sodium (Na), sulfur (S), calcium (Ca), magnesium (Mg), iron (Fe), boron (B), copper (Cu), manganese (Mn), and zinc (Zn) (Sah & Miller, 1992).

2.3 | Soil analyses

Soil pH was determined for all 900 samples and was measured in a 1:2 soil/deionized water ratio (McLean, 1982). Soils from the control transects were used to determine soil texture using the hydrometer method (Gee & Bauder, 1986) because 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 ˚C until mass was stable. Masses of soil samples were corrected for moisture using the gravimetric water content and ground finely using a mortar and pestle. Soil total C and N concentrations were measured on a Costech ECS 4010 CHNS-O Elemental Analyzer (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 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 C (SIC) (Harris et al., 2001). Acid-treated soils were analyzed on the Costech ECS 4010 CHNS-O Elemental Analyzer (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 at the UC Merced Stable Isotope Laboratory to determine the concentration of SOC. Soil inorganic C 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). 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 were then fitted with a cubic spline to determine SOC and total N mass per area.

2.4 | Microbial biomass

The chloroform fumigation method is an effective measure of how much C and N is associated with microbial biomass. We analyzed 3 out of 10 replicates per transect from the 0-to-10-cm and 10-to-30-cm depth intervals, where the majority of microbial activity occurs, for microbial biomass C (MBC) and microbial biomass N (MBN) (Fernandes et al., 2005). Soils were extracted within 24 h 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 C analyzer (TOC-Vcsh, Shimadzu) 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 nonfumigated 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).

2.5 | Rates of SOC sequestration

Rates of SOC sequestration were calculated for each paired transect at each site ($n = 9$) using three approaches. (a) We determined annual rates of SOC sequestration on a mass per area basis (e.g., Mg C ha^{-1} yr^{-1}) to a 30-cm depth. Differences in cumulative SOC from 0 to 30 cm between amended and control soils were calculated and divided by 20 yr. (b) The same mass per area of field site per year approach was used for the cumulative 0-to-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. (c) We determined rates of SOC sequestration based as a function of the total amount of biosolids applied. For each transect, average treatment differences in SOC were divided by the total amount of biosolids applied, resulting in a sequestration value in units of mass of C per mass of biosolids (e.g., Mg C per Mg biosolids).

2.6 | 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 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, nonparametric statistical analysis using the Kruskal–Wallis test was performed. Multiplicative and additive error propagation was determined for statistical analyses. Data that were >2 SD away from the mean were classified as outliers (Stephenson, 2003). Statistical significance was established a priori at $\alpha = .05$, and marginal significance ranged from α > .05 to α < .10. Data are reported as means ± SE.

Cumulative soil C content from 0 to 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-to-10-cm and the 10-to-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 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 the 0-to-10-cm and 10-to-30-cm depths. Microbial biomass/SOC ratios were calculated then multiplied by 100 to get MBC/SOC percentages as described in Sparling (1992).

3 | RESULTS

3.1 | Biosolids characterization

Despite the wide variation in daily flow rates and size of populations served by the 10 wastewater treatment plants, elemental composition of fresh biosolids samples was consistent. Nutrient composition of fresh biosolids samples was similar except for elemental S ($p = .04$; Supplemental Table S2). Carbon concentrations of fresh biosolids ranged from 338 to 443 mg kg^{-1}, and total N concentration of biosolids ranged from 51 to 75 mg kg^{-1}. Biosolid C/N ratios ranged from 6 to 7.

An estimated total of 125 ± 12 Mg biosolids-C ha^{-1} (359 ± 35 Mg biosolids$_{dry}$ ha^{-1}) was added in the Sacramento site over 20 yr. 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 3 or 5 yr at Solano and Merced, respectively). The total amount of C applied from biosolids over 20 yr was 26 ± 4 Mg biosolids-C ha^{-1} (74 ± 10 Mg biosolids$_{dry}$ ha^{-1}) in Solano and 36 ± 9 Mg biosolids-C ha^{-1} (105 ± 27 Mg biosolids$_{dry}$ ha^{-1}) in Merced (Table 1). During the 20-yr period, 19 ± 2 Mg biosolid-N ha^{-1} was applied at Sacramento, 4 ± 1 Mg biosolids-N ha^{-1} was applied at Solano, and 6 ± 1 Mg biosolid-N ha^{-1} 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.

3.2 | Soil pH and texture

Soils at the Sacramento and Solano sites were predominantly clay loam, whereas soils at the Merced site were sandy loam (Supplemental Table S1). Surface (0–10 cm) soil pH varied widely among the sites, ranging from 6 ± 0.1 (Solano) to 8 ± 0.2 (Merced) (Supplemental Table S1). 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 ($p = .4$) or Merced ($p = .1$). There was a marginally significant treatment effect at Solano ($p = .1$), where soils amended with biosolids had a pH of 7.0, compared with 6.6 in control soils.

3.3 | Soil C stocks

The percentages of C in the biosolids-amended and control soils were significantly different from each other in the Sacramento and Solano sites down to the 100-cm depth (both $p < .001$); however, no significant differences were observed between treatments in the Merced soils ($p = .6$). The Solano site experienced the greatest increase in SOC with biosolids amendment. At this site, cumulative SOC to 100 cm in biosolids-amended transects was 10 ± 1 Mg C ha^{-1} greater than control soils. Carbon stocks in biosolids-amended soils at the Sacramento site were approximately 5 ± 2 Mg C ha^{-1} greater than in control soils. The lowest amount of C sequestered was at the Merced site, with 3 ± 3 Mg C ha^{-1} 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 to 10 cm ($p < .001$) (Figure 1a). Biosolids application marginally increased SOC content of the 0-to-10-cm depth compared with the control soils at the Sacramento ($p = .10$) and Merced ($p = .1$) sites, and no significant differences were detected from 10 to 30 cm at any site.

At the Solano site, biosolids application significantly increased cumulative SOC by 50% ($p < .001$), with significant treatment effects observed in the 0-to-10-cm ($p < .001$), 30-to-50-cm ($p < .001$), 50-to-75-cm ($p = .004$), and 75-to-100-cm ($p = .001$) depth intervals. Cumulative SOC amounts to 100 cm in biosolids amended soils were 32 and 20% greater compared with controls in the Sacramento and Merced sites (not statistically significant). No depth increments from 30 to

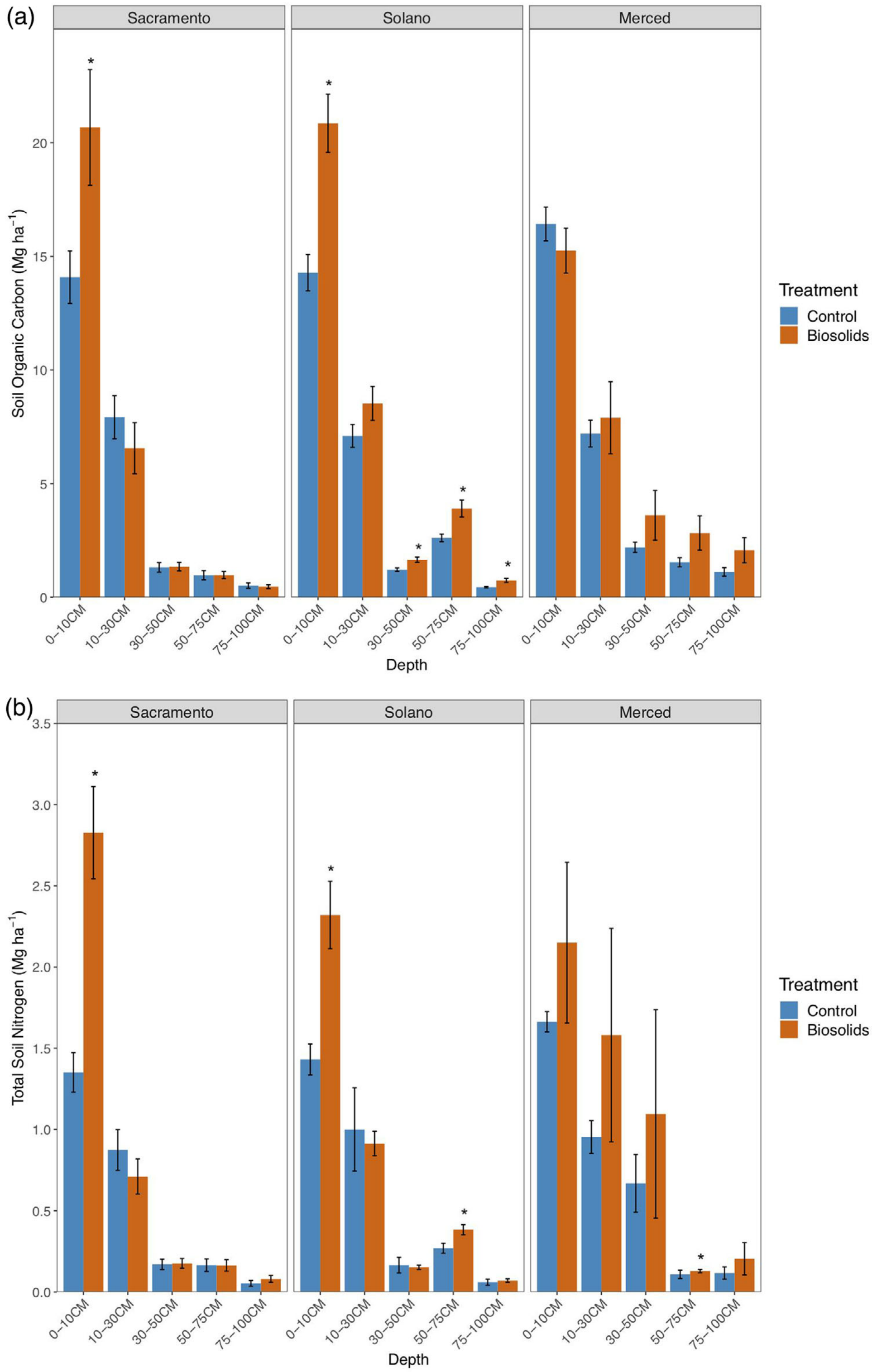

FIGURE 1 Mean values of (a) soil organic C and (b) soil total N after 20 yr of biosolids application in five depth increments to 1 m in three agricultural ecosystems. Asterisks indicate statistical significance between treatments ($p < .05$). Error bars are SE

50 cm, 50 to 75 cm, or 75 to 100 cm were significant at the Sacramento or Merced site.

Soil inorganic C was ∼22% of the total soil C across both treatments in the Merced site. Soil inorganic C in the unamended controls ranged from 0 to 120 Mg ha^{-1}. Biosolids-amended soils had SIC contents ranging from 0 to 104 Mg ha^{-1}. The wide ranges in SIC content suggest high spatial variability of the soil conditions at this site. There was no

TABLE 2 Sequestration rates using three methods

Site	Rate of C storage 0–30 cm	Rate of C storage 0–100 cm	Per biosolids applied (0–100 cm) Mg C per Mg biosolids
	———Mg C ha^{-1} yr^{-1}———		
Sacramento	0.3 ± 0.2	0.2 ± 0.2	0.0 ± 0.1
Solano	0.4 ± 0.1	0.5 ± 0.1	1.0 ± 0.2
Merced	−0.0 ± 0.2	0.2 ± 0.2	0.5 ± 0.4

Note. Values are site means ± SE ($n = 90$ samples per type of sequestration rate).

significant treatment effect on SIC between the biosolids-amended and unamended soils ($p = .2$); therefore, changes in SIC were not included in estimates of C sequestration per area or per amount of biosolids applied.

3.4 | 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 at the Sacramento site when expressed as a function of mass of biosolids applied but were higher at the Sacramento site compared with the Merced site when expressed on an areal basis (i.e., Mg C ha^{-1}; area of field sites) from 0 to 30 cm. If we assume linear rates of C sequestration over the 20-yr period, annual rates of C sequestration to a 30-cm depth were approximately 0.4, 0.3, and −0.04 Mg C ha^{-1} yr^{-1} at the Solano, Sacramento, and Merced sites, respectively (Table 2). Negative values indicate less C at the 0-to-30-cm depth in the biosolids treated transects compared with the control transects. When considering a 0-to-100-cm soil depth, these values increase to 0.5 (Solano) and 0.2 (Merced) Mg C ha^{-1} yr^{-1}, except in Sacramento where the C rate was estimated to be 0.2 Mg C ha^{-1} yr^{-1}. When expressed as changes in mass of soil C per mass of biosolids applied, average C sequestration rates were 1 ± 0.2 in Solano, 0.04 ± 0.1 in Sacramento, and 0.5 ± 0.4 in Merced. These rates were marginally significant from 0 at the 0-to-100-cm depth across all sites ($p = .10$).

3.5 | 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 ($p < .001$) and from 1 ± 0.1 to 3 ± 0.3 Mg N ha^{-1} in the Sacramento site ($p < .001$) (Figure 1b). The Merced site experienced no significant changes in soil total N from 0 to 10 cm ($p = .3$). 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 biosolids-amended soils at the Sacramento site ($p < .001$) 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 ($p < .001$) from 0 to 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 with control soils (3 ± 0.1 Mg N ha^{-1}); this change was not statistically significant ($p = .3$). Down to 100 cm depth, soil total N increased from 4 ± 0.1 to 5 ± 0.2 Mg N ha^{-1} ($p = .02$) in Sacramento and from 3 ± 0.1 to 4 ± 0.1 Mg N ha^{-1} ($p < .001$) in Solano. Similarly, the application of biosolids increased total N in Merced soils by 35%, though this increase was marginally significant (5 ± 0.2 Mg N ha^{-1} biosolids; 4 ± 0.1 Mg N ha^{-1} control) from 0 to 100 cm ($p = .06$).

3.6 | Microbial biomass

At the Sacramento site, MBC from 0 to 10 cm in biosolids-amended soils was 656 ± 44 mg C kg^{-1}, compared with 280 ± 41 mg C kg^{-1} in the control ($p < .001$) (Figure 2a). Biosolids-amended soils from 10 to 30 cm in the Sacramento site also increased from 66 ± 8 to 150 ± 15 mg C kg^{-1} ($p < .001$). At the Solano site, microbial biomass C increased in amended soils from 234 ± 37 to 378 ± 60 mg C kg^{-1} at the 0-to-10-cm depth ($p = 0.2$) and from 48 ± 7 to 163 ± 40 mg C kg^{-1} at the 10-to-30-cm depths ($p = .02$). The ratio of microbial biomass to SOC (MBC/SOC) was significantly higher in biosolids-amended soils at the Solano site ($p = .01$) but was not significant at the Sacramento ($p = .2$) and Merced sites ($p = .1$) (Figure 2b).

Trends in MBN followed similar patterns as MBC. Biosolids application significantly increased MBN compared with the control by 79% from 0 to 10 cm and 114% from 10 to 30 cm in the Sacramento site (both $p < .01$). No increase was observed in microbial biomass N from 0 to 10 cm and from 0 to 30 cm at the Solano site ($p = .4$; 0.3). Significant treatment effects in MBN were not observed at either depth at the Merced site ($p = .1$) (Figure 2c).

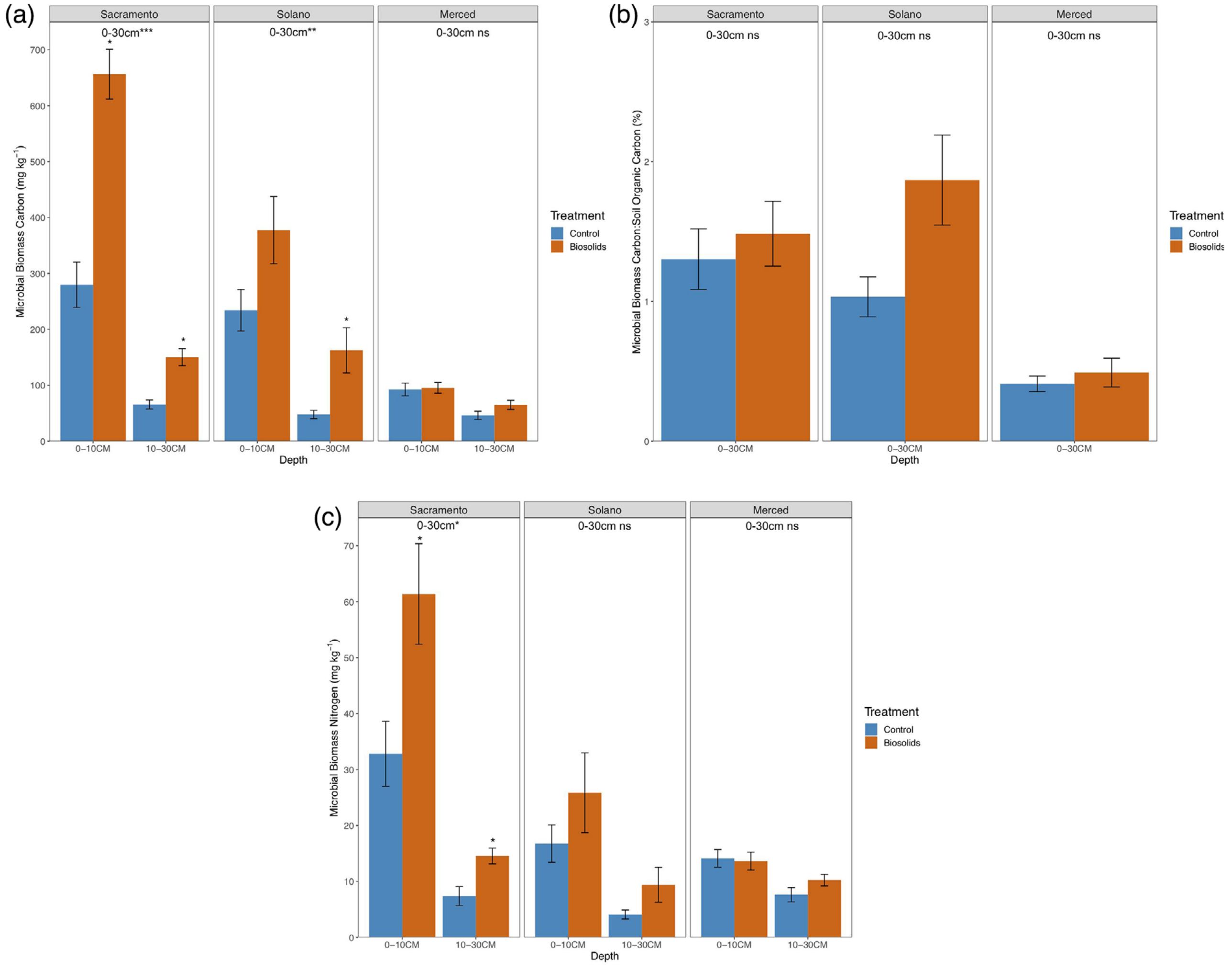

FIGURE 2 Changes in microbial biomass. (a) Carbon. (b). Microbial biomass C to soil organic C ratio percentages. (c) Microbial biomass N separated by depth and down to 30 cm. Asterisks indicate statistical significance (*$p < .05$; **$p < .01$; ***$p < .001$; ns, $p > .05$)

4 | DISCUSSION

4.1 | 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 noncomposted biosolids promoted organic C protection in microaggregates, whereas composted biosolids promoted organic C 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 at 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 was 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 yr of application.

4.2 | Biosolids effects on SOC stocks

Amendments are a direct source of C; some of this C may persist in soils, and some is lost through decomposition and respiration (Ryals et al., 2014). In our study, long-term (20-yr) 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 improve 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 is one of the most important and interrelated parameters and serves as the basis for some of the most important soil processes, such as N and P cycling; however, it is also an indicator of a soil's resilience to climate change and management practices (Lal, 2014).

Assuming the initial concentrations of C in the control soils were similar to the biosolids-amended soil before biosolids were applied 20 yr ago, soil stocks from 0 to 100 cm were 23 ± 3 Mg C ha^{-1} at the Sacramento site, 25 ± 2 Mg C ha^{-1} at the Solano site, and 28 ± 2 MgC ha^{-1} at the Merced site. From 0 to 100 cm, the biosolids treatment soil C was 27 ± 4 Mg C ha^{-1} at Sacramento, 36 ± 2 Mg C ha^{-1} at Solano, and 36 ± 2 Mg C ha^{-1} at Merced. A significant change from the control soil C to the biosolids-treated soil occurred at the Solano site, indicating that the rate of C sequestration there is faster or that 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 that 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 because this site received the least cumulative amount of biosolids over the 20-yr 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 with the Solano site. Despite intensive biosolids use at Sacramento, increases in SOC to 100 cm (5 ± 2 Mg C ha^{-1}) were about half of the observed changes at the Solano site. Further, increases in SOC were restricted to the top 10 cm of soil, whereas no significant differences were observed in any depth interval from 10 to 100 cm. Flood irrigation can cause a pulse of CO_2 from microbes, which causes C losses (Guo et al., 2017). Although 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 & Piccolo, 2020). One of the paired transects was classified as a Mollisol, which is known to be a soil of higher organic matter in the surface layer; no large variation in SOC was observed from 0 to 30 cm in the control soils compared with the other two 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 were most likely due to agricultural practices, such as tillage and flood irrigation. We observed no treatment difference in SOC to the 30-cm depth 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 applications and lower application rates of biosolids can also inhibit accumulation of organic C (Hamdi et al., 2019).

4.3 | 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 yr of 0.5 Mg biosolids-C ha^{-1} (Merced), 1 Mg biosolids-C ha^{-1} (Solano), and 0.04 Mg biosolids-C ha^{-1} (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-to-15-cm soil depth and no change from 15 to 30 cm (Brown et al., 2011). A more recent study found rates as high as 1 Mg C Mg^{-1} dry biosolids in the top 10 cm of soil (Pan et al., 2017). 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 Mg^{-1} dry biosolids applied) is greater than other published values (Brown et al., 2011; Pan et al., 2017). Both the Sacramento and Solano sites are annual grasslands managed for rotational cattle grazing, but Sacramento is flood irrigated, whereas Solano is rainfed. Frequent flood irrigation is used at the Sacramento site, whereas 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 with 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 transects was classified as a Mollisol, which tend to have higher SOC content compared with 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-to-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 leads to a 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, leading to misinterpretation of 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 of the lack of estimated turnover times of this C pool (Gravuer et al., 2019).

4.4 | 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 with 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 & Brady, 2017). However, a previous field study found that after 3 yr 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; and grew corn, which has a high N uptake, suggesting that irrigation practices influence total N retention in soil instead of soil texture.

Nicolás et al (2014) and Pan et al. (2017) concluded that application rate may be the reason they found an increase in total N in their studies compared with 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 the low frequency of biosolids application and rates, the coarser soil texture, and management.

4.5 | 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 at al. (2004) showed an increase of microbial biomass C in the top 10 cm of soil in ryegrass (*Lolium perenne* L.)–clover (*Trifolium repens* L.) pasture with the soil texture as the Merced site after only 4 yr of annual applications. Other studies have shown an increase in MBC when the rate of biosolids application is increased, indicating application rate determines changes in microbial activity (Fernandes et al., 2005; Hamdi et al., 2019).

The 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 results between both agroecosystems. Low application rates, coarse soil texture, and high inorganic C may contribute to unfavorable environments for microbes (Mavi et al., 2012).

Microbial biomass N is a good measure of mineralizable N because 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).

5 | CONCLUSIONS

The current results show that, despite high yearly application rates, C sequestration at the Sacramento site was the second highest among 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 influence C storage or that 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: 0–100 cm, 0.53 Mg C ha^{-1} yr^{-1}; 0–30 cm, 0.40 Mg C ha^{-1} yr^{-1}). Further application of biosolids at these sites may reveal further information about the ability of the soils to sequester C and the extent to which a soil can be enriched by C or at which concentration the soil will reach a saturation point. In addition, determining where C is in different pools and 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 are needed to determine optimal agricultural context for biosolids reuse.

ACKNOWLEDGMENTS

Funding for this research was provided by a grant to R. Ryals from the Bay Area Clean Water Agencies and the Jena and Michael King Foundation. Funding was also provided by the Department of Life and Environmental Sciences at the University of California, Merced. We thank F. Gamiño, E. Phosalth, B. Alrawi, C. De Leon, K. Porterfield, S. Cousineau, N. Goncalves, D. Davenport, and Ryan Batjiaka; our farming partners; and members of the San Francisco Public Utilities Commission; and the two anonymous reviewers who helped improved the quality of this manuscript.

AUTHOR CONTRIBUTIONS

Yocelyn B. Villa: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing. Rebecca Ryals: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing-review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Villa, Y. B., & Ryals, R. (2021). Soil carbon response to long-term biosolids application. *J Environ Qual*, 1–13. https://doi.org/10.1002/jeq2.20270