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Carbon persistence of soils with long-term biosolids amendments in California agroecosystems

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Abstract

Biosolids can build soil organic matter, but their ability to increase carbon and nitrogen in persistent fractions in deep soil is not well understood. We aimed to assess the mechanisms that influence soil carbon and nitrogen dynamics at three sites—Sacramento (irrigated, grazed grassland), Solano (rainfed, grazed grassland), and Merced (feed cropping system with alfalfa–corn rotation)—where soils were amended with biosolids for 20 years using density fractionations, organomineral extractions, and correlations between calcium and soil organic carbon at three depths (0–10, 30–50, and 75–100 cm). We found that amended soils had higher carbon and nitrogen content in the free- and occluded-light fractions at all depths relative to the control in the Sacramento and Solano sites; however, the Merced site had a greater relative increase of carbon and nitrogen associated with the heavy fraction. Effect sizes show that biosolids increase carbon and nitrogen content in free- and occluded-light fractions in the surface soil (0–10 cm), and in both light and heavy fractions in the deep soil layer (75–100 cm). Ratios of carbon to iron and aluminum show that chelation is an important mechanism of carbon stabilization in Sacramento and Solano sites throughout the soil profile. No (0–10 cm) to negative (75–100 cm) correlations were observed between calcium and soil carbon in the amended soils in the Merced site. Our results indicate that, while biosolids are typically incorporated at shallow depths, long-term application of biosolids can increase the amount of free- and occluded-light carbon fractions in deep soil.

1 | INTRODUCTION

Management-induced soil carbon (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 & Huntsinger, 2021; Deen & Kataki, 2003; Mazzoncini et al., 2011; Powlson et al., 2012; Ryals et al., 2014). Application of organic matter amendments has been proposed as a way to increase soil organic carbon (SOC) stocks directly through the addition of persistent 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

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Abbreviations: FLF, free-light fraction; HF, heavy fraction; OLF, occluded-light fraction; SIC, soil inorganic carbon; SOC, soil organic carbon; SOM, soil organic matter; TOC, total organic carbon.

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affecting the magnitude of changes to soil organic matter (SOM) and the potential benefit of this practice.

The production of biosolids has increased, 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 their 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 SOC, which in turn increases and improves soil fertility (Brown et al., 2011; Charlton et al., 2016; Cogger et al., 2013; Nicholson et al., 2018; Villa & Ryals, 2021). Although long-term biosolids application has been shown to provide benefits for soil fertility and increasing SOC 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 organomineral complexes that can contribute to the persistence of soil C over long periods of time (Kögel-Knabner et al., 2008; Poeplau & Don, 2015; Tautges et al., 2019; Villa & Ryals, 2021; Yost & Hartemink, 2020). There have been some studies that have focused on biosolids application and assessing the persistence of these carbon pools through fractionation techniques; however, these studies sampled soils in the top 30 cm of soil (Chiu & Tian, 2011; Pan et al., 2017; Silva et al., 2015; Wijesekara et al., 2017). Thus, there is little information on assessing how biosolids application may influence C sequestration of soils, specifically the soil dynamics that may play a role in the persistence of that C. Understanding these mechanisms would provide insight into the vulnerability of C and how to properly manage these soils to get the maximum benefits in terms of soil fertility and climate benefits (Tautges et al., 2019; Wang et al., 2021).

Deep soil contains about 68% of global SOC from 30 to 200 cm (Jackson et al., 2017). Research has shown that deep soil C may also be mobile under certain conditions, indicating that studying topsoil is no longer sufficient to understand how soils respond to climate change (Berhe et al., 2008). The magnitude and direction of deep soil C changes with management and global change remain understudied (Hicks Pries et al., 2017, 2023; Soong et al., 2021). Neglecting to sample deep soil C can lead to misleading conclusions on the climate change mitigation potential of soil (Harrison et al., 2011).

The rate at which SOC is mineralized to the atmosphere is determined by soil physiochemical stabilization mechanisms of 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 improve our ability to determine how management-induced changes influence pools of C and nitrogen (N) and provide an indication of the physical location of C and N in the soil matrix and how easily mineralizable

Core Ideas

- Long-term biosolids increased deep soil C and N concentrations.
- Density fractionation indicates amended soils had more labile C and N in surface soils.
- Organometal complexes were a stabilization mechanism in two of the three sites.
- Negative correlations between C and Ca^{2+} in amended soils indicate biosolids interact with C.
- Accounting for deep soil C demonstrated the importance of assessing the climate change mitigation potential of managed soil.

C and N are to microbes (Powlson et al., 2012; Ryals et al., 2014; Swanston et al., 2005). For this study, bulk soil C and N were 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 mineral-associated heavy fraction (HF).

In addition, current understanding suggests other physicochemical parameters may be better predictors than clay content in order to determine the stability of SOC (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 the persistence of SOC (Kaiser & Guggenberger, 2000; Rasmussen et al., 2006). Rasmussen et al. (2018) synthesized over 5500 soil profiles and confirmed that other soil physicochemical properties predict SOM stabilization better than clay. They found that soil pH has an important, but complicated, influence on SOM content (Rasmussen et al., 2018). Soil pH reflects the overall chemical state of soil, which dictates the speciation of dissolved metals, the reactivity and charge of minerals, organic molecules, and the dominant type of organomineral type in soils (Rasmussen et al., 2018). Rasmussen et al. (2018) also found that a high soil pH (>6.5) corresponded to high concentrations of calcium, while a lower pH (<6.5) corresponded to high concentrations of extractable aluminum (Al).

Polyvalent cations, such as calcium (Ca^{2+}), can play a significant role in the stabilization of SOC by creating bridges between organic compounds and mineral surfaces (Rasmussen et al., 2018; Rowley et al., 2021; Solly et al., 2020). In addition, Ca^{2+} is also thought to indirectly contribute to SOC associated with OLF by promoting soil aggregation through the bridging of organic matter to calcium (Oades, 1988; Rowley et al., 2018, 2021). Biosolids contain high levels of iron (Fe) and aluminum (Al) due to industrial wastewater sources and/or the treatment process (Tian et al.,

2013). Hence, studies have shown that long-term applications of biosolids could increase soil amorphous Fe and Al, leading to increased levels of SOC stability through the formation of organomineral complexes (Chen et al., 2019; Chiu & Tian, 2011; Silva et al., 2015; Tian et al., 2013; Wang et al., 2021). The properties of these organic materials, such as Fe and Al concentrations, may raise some additional questions and challenges on how to properly manage them. Hence, it is important to understand how biosolids influence C permanence in agricultural soils with different soil physicochemical properties, such as soils with varying pH or a high concentration of Ca^{2+}.

We determined the changes in the amount and distribution of C in soil fractions from three agroecosystems that have received 20 years of biosolids application. We assessed these changes in the surface soil (0–10 cm), as well as in subsoil layers (30–50 and 75–100 cm). We also evaluated potential mechanisms that influence soil C dynamics across these fractions, including organomineral associations at all sites (referred to as Merced, Sacramento, and Solano) and Ca^{2+} concentrations in the HF at the site with high pH (Merced). We hypothesized that (1) the application of biosolids C and N would increase C and N present in the FLF in surface soil across the three sites. (2) Treatment effects on C and N will be dependent on fractions and depth, where less persistent fractions would have positive effects at the surface soils and more persistent fractions would have positive effects in deeper soil depths where accumulation of minerals is typically found. (3) Both amended and control soils from sites that have a lower pH will have stronger organomineral C content than the sites 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 high pH and concentrations of calcium carbonate ($CaCO_3$).

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; Figure S1). Briefly, 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. Neither the biosolids treatment nor the control received inorganic fertilizer. Biosolids were applied at each site according to local regulations for biosolids land application. Detailed soil characteristics of the three sites can be found in Tables S1 and S2. The Sacramento site soils are classified as Alfisols from the Hicksville and Corning series. This site consisted of flood-irrigated annual

TABLE 1 The chemical composition of C, N, C:N, and Fe of biosolids from 10 wastewater treatment plants.

Nutrients	Biosolids composition
C ($mg \cdot kg^{-1}$)	381.2 ± 32.6 (338–443)
N ($mg \cdot kg^{-1}$)	58.3 ± 9.5 (49.2–74.7)
C/N	6:1 (5:1–7:1)
Fe ($mg \cdot kg^{-1}$)	34.8 ± 19.2 (7.3–64)

Note: The data presented are on a dry weight basis; values were averaged; standard deviations are shown. Values in brackets are the minimum and maximum value. One-way analysis of variance was used to determine significant differences as described in Villa and Ryals (2021). The biosolids composition of the elements below was not statistically different from each other across the wastewater treatment plants ($p < 0.05$).

grasslands managed for grazing beef cattle. Livestock was rotated every 60–70 days depending on feed availability. Soils at the Solano site are classified as Alfisols from the Antioch and Pescadero series and Vertisols from the Altamont soil series. The Solano site consists of rainfed annual grasslands used for grazing lambs and beef cattle. Livestock are rotated seasonally and based on feed availability. The Merced site soils are classified as Mollisols from the Pozo series and Alfisols from the Fresno series. The site is flood irrigated and managed for livestock feed crops consisting of 1-year corn and 4-year alfalfa rotations for livestock silage and was tilled after each rotation. Sampling at these sites consisted of establishing an unamended control and an amendment treatment where biosolids were applied for 20 years as described in detail in Villa and Ryals (2021). Biosolids applied across all sites were dewatered and classified as Class B biosolids (Table 1). County-level regulations on biosolids application are prohibited within a predetermined distance from certain areas, including property lines, roadways, and water supply wells. We selected sites that included a comparative unamended area, referred to as "control," immediately adjacent to the area where biosolids were applied (Villa & Ryals, 2021). The rate and frequency of biosolids application varied across sites (Table S3). At all sites, 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. 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, 10–30, 30–50, 50–75, and 75–100 cm) at every 10 m along a 100-m transect ($n = 10$). To obtain a valid comparison of SOC and total N stocks, we used the equivalent soil mass method (Ellert & Bettany, 1995; von Haden et al., 2020; Wendt & Hauser, 2013). Soil mass was calculated by dividing the dry soil sample mass of the depth layer by the area of the sampled auger. Then,

the mass of the soil was multiplied by the SOC or total N concentration of that soil sample layer. These data were then fitted to a cubic spline to determine SOC and total N mass per area (Wendt & Hauser, 2013). 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. Total organic carbon (TOC) and total N concentrations were measured for all 900 samples as described in Villa and Ryals (2021). Soils were tested for effervescence upon the addition of 4 N HCl, indicating the presence of soil inorganic carbon (SIC) (Harris et al., 2001). Effervescence was only detected at the Merced site where SIC accounted for ~22% of the total C (Villa & Ryals, 2021). One paired transect (amended treatment and an unamended control) with the highest relative change was selected per site for density fractionations ($n = 3$).

2.2 | Density fractionations

A subset of sampled depth intervals (0–10, 30–50, and 75–100 cm) from each paired transect were selected for analysis with density fractionation due to the high cost and time required for this method. Samples were density separated at 1.85 g·cm^{-3} as described in Swanston et al. (2005), which separates bulk soil into three fractions to provide information on the potential turnover times of C and N in soils. The organic matter in the FLF closely resembles plant material that is "free floating" in the soil matrix and not protected by mineral surfaces. This material is more easily mineralizable to microbes and often has the fastest turnover time (Marín-Spiotta et al., 2008; Swanston et al., 2005). The OLF contains C and N that are physically protected in soil aggregates. This makes it more difficult for decomposers to access it; hence, this fraction tends to have a slower turnover time than that of FLF. The HF contains C and N that are bound to mineral surfaces such as clay and metals. Typically, HF has the longest residence time compared to FLF and OLF.

Briefly, 20 g of soil was gently mixed with 75 mL of sodium polytungstate prepared to a density of 1.85 g·cm^{-3}. After centrifuging for 1 h at 3500 rpm on an 18-cm-radius centrifuge rotor, the FLF was aspirated off, filtered through a 0.8-μm polycarbonate membrane filter (Whatman Nuclepore Track Etch Membrane), rinsed with deionized (DI) water five times, dried at 50°C for 48 h, and then weighed. The remaining sample was then mixed, sonicated at 70% pulse for 3 min, and then centrifuged for 1 h at 4000 rpm. The OLF was aspirated off, filtered, rinsed five times with DI water, dried at 50°C for 48 h, and then weighed. The remaining soil pellet, known as the HF, was mixed with DI water, centrifuged five times, dried at 150°C for 24 h, and then weighed.

2.3 | Total C and N of fractions

Fractions were analyzed for TOC and total N on the Costech ECS 4010 CHNS-O Elemental Analyzer coupled to a Thermo Scientific Delta-V Plus continuous flow isotope ratio mass spectrometer at the Stable Isotope Ecosystem Laboratory of UC Merced. The HF from the Merced site was treated further using the acid fumigation method due to the presence of inorganic C (Harris et al., 2001). Acid-treated HFs were analyzed on the elemental analyzer to determine the concentration of SOC. SIC was calculated by subtracting the acid-treated C concentrations from total soil C concentrations.

2.4 | Organometal complexes in HFs

The HF was used for organomineral extractions following Heckman et al. (2018). Briefly, 40 mL of 0.1 M sodium pyrophosphate was added to 1 g of air-dried ground sample and shaken for 16 h. Then, samples were centrifuged at 20,000 rpm on an 18-cm-radius centrifuge for 20 min. 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 with DI at a 1:50 ratio and analyzed on an Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) for Fe and Al at the UC Davis Analytical Laboratory.

2.5 | 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 & Quick, 1960). This extraction procedure was only done for soils in the Merced site since inorganic C was not detected at the Sacramento and Solano sites.

2.6 | Statistical analyses

The results of this study are presented as treatment means (± standard errors). Shapiro–Wilk tests were used to determine the normality of data for all analyses. Tukey's post hoc tests were used for determining interactions between factors. A three-way repeated measures analysis of variance (ANOVA) was performed on density fraction samples and organometal extractions between depths to determine the effects of treatment, fraction, and site between depths. The determination of contributions of TOC and total N from each

fraction was assessed by dividing the C and N by the total for each depth within a site. Treatment effects were analyzed using a two-way ANOVA with treatment and fractions as factors. We used a Pearson's correlation (r) test to determine if SOC% and Ca^{2+}, Fe, and Al were correlated in the HF. The Pearson coefficient (R^2) quantifies a correlation: a positive value closer to 1 indicates a strong correlation, 0 signifies no correlation, and negative correlations (closer to -1.0) represent a strong inverse relationship. The p-values from the correlation test demonstrate whether the correlation is statistically significant. Effect sizes of C and N content in fractions were calculated by taking the average of the percent change across sites within a treatment, depth, and fraction. The average of the biosolids' percent change was divided by the control's percent change, and the natural log was taken of that value as shown in the equation below:

$$\text{Effect size} = \ln\left(\frac{X_b}{X_c}\right),$$

where X_b is the average of the percent change of the biosolids treatment and X_c is the average percent change of the control treatment. Statistical tests were performed using RStudio 3.3.1.

3 | RESULTS

Our results demonstrated how management-induced changes influence soil C stock at deeper soil depths. Overall, soils amended with biosolids have shown increases in SOC and N down to a depth of 1 m, where they can persist for longer durations compared to surface soils. Interestingly, in the majority of cases, the differences in soil C and total N content in the biosolids and unamended soils are drastically different. Proportions of fractions contributing to the total C and N content varied based on treatment, depth, and site (Tables S4 and S5). Biosolids application increased the amount of C and N content in the FLF relative to the control in the Sacramento and Solano sites (Tables 2 and 3). In contrast, biosolids-amended soils at the Merced site had more C and N content associated with the HF across all depths. Control soils had more C and N content associated with the OLF compared to the biosolids-amended soils, where most of the total C and N content is associated with the FLF, indicating that biosolids application adds more C and N content that is attributed to FLF. Here, we represent the results of the mass distribution of C and N among soil fractions and the treatment effect size with a focus on depth increment,

3.1 | Mass distribution of C and N among fractions and depths

3.1.1 | The 0–10 and 30–50 cm depths

The trends observed in mass C and N distribution between 0–10 and 30–50 cm were similar. Both depths had no significant differences in total SOC content observed between treatments (0–10 cm, $p = 0.36$; 30–50 cm, $p = 0.69$) or sites (0–10 cm, $p = 0.97$; 30–50 cm, $p = 0.75$). However, there was a significant effect of fractions on C content across all sites (both depths $p < 0.001$; Figure 1a,b). There was a significant interaction between treatment and site in the 0–10 cm depth ($p = 0.01$). Similar to C, total N content in fractions from 0–10 and 30–50 cm was not significantly different between sites (0–10 cm, $p = 0.43$; 30–50 cm, $p = 0.96$) or treatments (0–10 cm, $p = 0.52$; 30–50 cm, $p = 0.91$; Figure 2b), but significant differences in N contents were observed between fractions (0–10 cm, $p < 0.001$; 30–50 cm, $p < 0.01$; Figure 2a). Significant differences in mass of C and N content were observed in fractions ($p < 0.01$), but no interactions were observed between factors from 30–50 cm. However, from the 0–10 cm depth, interactions among these factors were observed: treatment and site ($p = 0.02$), treatment and fraction ($p = 0.01$), and all three factors of treatment, site, and fraction ($p < 0.001$).

3.1.2 | The 75–100 cm depth

The C content in the deep soil layer (75–100 cm) showed significant differences between treatment and fractions ($p = 0.01$; $p < 0.001$) with a marginal significance between sites ($p = 0.08$; Figure 1c). The total N content at the soil depth of 75–100 cm had marginally significant treatment ($p = 0.09$) effects, with significant site ($p < 0.001$) and fraction ($p < 0.001$) differences; no interactions were observed between factors (Figure 2c).

3.2 | Treatment effects of biosolids application on C and N

3.2.1 | Effect size

The effect size demonstrates the strength of the relationship between C content and biosolids application, where positive effect size values indicate that biosolids application increases C, negative values indicate a C loss with biosolids application,

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TABLE 2 Treatment differences between depth, fraction, and site in (a) carbon and (b) nitrogen.

Site	Treatment	Depth	Carbon			Nitrogen		
			FLF (Mg C·ha^{-1})	OLF (Mg C·ha^{-1})	HF (Mg C·ha^{-1})	FLF (Mg N·ha^{-1})	OLF (Mg N·ha^{-1})	HF (Mg N·ha^{-1})
Sacramento	Biosolids	0–10	22.00 ± 5.86	2.29 ± 0.29	1.38 ± 0.38	2.47 ± 0.47	0.37 ± 0.09	0.16 ± 0.03
	Control		7.84 ± 0.22	8.86 ± 1.44	3.84 ± 0.21	0.80 ± 0.19	0.70 ± 0.10	0.53 ± 0.04
Solano	Biosolids	0–10	21.61 ± 1.18	8.95 ± 7.35	4.11 ± 0.61	3.29 ± 0.40	0.72 ± 0.59	0.62 ± 0.08
	Control		8.45 ± 3.77	1.85 ± 0.31	5.53 ± 0.30	0.73 ± 0.33	0.18 ± 0.04	0.77 ± 0.05
Merced	Biosolids	0–10	1.55 ± 0.76	3.56 ± 1.13	7.74 ± 2.89	0.19 ± 0.13	0.40 ± 0.23	0.95 ± 0.15
	Control		10.18 ± 8.71	4.87 ± 0.53	4.83 ± 0.39	1.80 ± 1.66	0.88 ± 0.21	0.66 ± 0.03
Sacramento	Biosolids	30–50	16.76 ± 14.46	12.82 ± 5.24	6.12 ± 2.38	1.08 ± 0.73	0.92 ± 0.55	1.03 ± 0.39
	Control		6.34 ± 3.74	9.29 ± 5.28	17.49 ± 13.33	0.47 ± 0.29	0.64 ± 0.33	3.44 ± 3.30
Solano	Biosolids	30–50	58.10 ± 39.64	1.96 ± 0.63	8.78 ± 1.59	6.10 ± 1.94	0.16 ± 0.05	1.05 ± 0.08
	Control		3.40 ± 1.40	1.73 ± 0.37	7.37 ± 1.54	0.38 ± 0.12	0.16 ± 0.05	1.65 ± 0.36
Merced	Biosolids	30–50	2.64 ± 1.16	1.41 ± 0.39	19.39 ± 4.57	0.31 ± 0.12	0.21 ± 0.09	1.20 ± 0.13
	Control		8.73 ± 8.58	10.94 ± 7.81	10.49 ± 2.01	2.09 ± 1.62	2.44 ± 1.87	1.50 ± 0.27
Sacramento	Biosolids	75–100	2.97 ± 1.49	3.46 ± 1.15	3.84 ± 1.18	0.36 ± 0.06	0.23 ± 0.06	0.61 ± 0.15
	Control		0.18 ± 0.05	1.27 ± 0.10	3.13 ± 0.44	0.01 ± 0.00	0.21 ± 0.03	0.60 ± 0.12
Solano	Biosolids	75–100	10.65 ± 9.57	21.71 ± 21.34	6.82 ± 0.92	1.09 ± 0.94	1.62 ± 1.59	1.22 ± 0.25
	Control		1.31 ± 0.36	1.88 ± 0.92	5.64 ± 0.90	0.17 ± 0.06	0.17 ± 0.06	1.22 ± 0.25
Merced	Biosolids	75–100	0.83 ± 0.24	0.58 ± 0.20	15.79 ± 11.06	0.12 ± 0.05	0.10 ± 0.04	0.96 ± 0.22
	Control		0.92 ± 0.25	0.56 ± 0.32	5.74 ± 0.71	0.14 ± 0.05	0.12 ± 008	0.80 ± 0.12

Abbreviations: FLF, free-light fraction; HF, heavy fraction; OLF, occluded-light fraction.

and zero represents no treatment effect (Figure 3). Positive treatment effects are present between fractions and depths in many cases, indicating shifts in the presence of SOC in the soil depending on the site and depth (Figure 4). When assessing the effect size from compiling C content associated with fractions across all sites, we found that biosolids application increases C in the FLF across all depths (Figure 3a). Effect sizes on N follow a similar trend as C, where the FLF has increased across all depths (Figure 3b).

3.2.2 | The 0–10 cm depth

Soil from 0 to 10 cm at the Sacramento site showed significant treatment effects in all three fractions where the FLF increased from 7.84 ± 0.22 to 22.00 ± 5.86 Mg C·ha^{-1} ($p = 0.03$);

however, both the OLF (control: 8.86 ± 1.44 Mg C·ha^{-1}, biosolids: 2.29 ± 0.29 Mg C·ha^{-1}) and HF (control: 3.84 ± 0.21 Mg C·ha^{-1}, biosolids: 1.38 ± 0.38 Mg C·ha^{-1}) decreased significantly (both $p < 0.001$; Figure 4a). No significant differences across fractions, treatments, or depths were observed at the Solano site from 0 to 10 cm (Table 2). The only significant treatment effects at the Merced site from 0 to 10 cm were observed in the FLF where the application of biosolids decreased FLF C to 1.55 ± 0.76 Mg C·ha^{-1} from 10.18 ± 8.71 Mg C·ha^{-1} in the control ($p = 0.01$; Table 2). From 0 to 10 cm at the Sacramento site, the FLF experienced an increase in treatment effect for total N ($p = 0.01$; Table 2), but a significant loss of 48% in the OLF ($p = 0.07$) and 69% in the HF ($p < 0.001$; Figure 5a). Marginal significance was shown in the FLF at the Solano site from 0 to 10 cm ($p = 0.05$; Table 2).

TABLE 3 Concentrations of total organic carbon (TOC), Fe, and Al from pyrophosphate extractions.

Site	Treatment	Depth (cm)	Al (mg·g⁻¹)	Fe (mg·g⁻¹)	TOC (mg·g⁻¹)	TOC/Fe	Pearson correlations	TOC/Al	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

Note: Ratios of TOC/Fe and TOC/Al and Pearson correlations are shown. NA values are due to undetectable concentrations of Fe or Al.

*$p < 0.05$; ***$p < 0.001$.

3.2.3 | The 30–50 cm depth

At the Solano site, only the FLF from 30 to 50 cm showed a considerable significant increase of 1609% (control: 3.40 ± 1.40 Mg C·ha⁻¹, biosolids: 58.10 ± 39.64 Mg C·ha⁻¹; $p = 0.09$; Figure 4b). At the Merced site, the HF increased by 85% (control: 10.49 ± 2.01 Mg C·ha⁻¹, biosolids: 19.39 ± 4.57 Mg C·ha⁻¹). However, the HF increased significantly at the Merced site from 5.74 ± 0.71 Mg C·ha⁻¹ in the control to 15.79 ± 11.06 Mg C·ha⁻¹ in the amended soils. The Solano site was the only site with a marginally significant increase of 1526% of total soil N content in the FLF ($p = 0.06$), but the Merced site experienced a loss of 85%, though not significant. There was no treatment effect in the HF from 75 to 100 cm across the three sites (all $p = 0.99$).

3.2.4 | The 75–100 cm depth

The magnitude of the positive treatment effect on C in fractions increased with depth. In the deepest soil layer sampled (75–100 cm), all soil fractions had positive effect sizes. Treatment effects at both Sacramento and Solano sites demonstrated remarkable increases in the C content in the deeper soil depth of 75–100 cm in the FLF (Sacramento: 1531%, $p < 0.001$; Solano: 712%, $p = 0.90$) and OLF (Sacramento: 172%, $p = 0.04$; Solano: 1054%, $p = 1.00$). From 75 to 100 cm,

total N has shown a significant increase of 2820% in the FLF at the Sacramento site (control: 0.01 ± 0.00 Mg N·ha⁻¹; biosolids: 0.36 ± 0.06 Mg N·ha⁻¹; $p < 0.001$). The Merced site decreased in the FLF and OLF from 75 to 100 cm except in the HF where it increased from 0.80 ± 0.12 Mg N·ha⁻¹ to 0.96 ± 0.22 Mg N·ha⁻¹ (Figure 5c).

3.3 | Correlations of organomineral complexes

At the Sacramento site, TOC was positively associated with Fe and Al at all depths, though not significant (all $p > 0.05$; Table 3). Only from 75 to 100 cm was there a negative association between Fe and TOC, though not significant ($r = -0.397$, $p = 0.74$). Biosolids-amended soils at the Sacramento site had stronger correlations between TOC and Fe and Al from 0 to 10 and 30 to 50 cm, though not significant except for Al from 30 to 50 cm ($p = 0.04$). From the 75–100 cm depth, negative correlations were observed for both Fe and Al associated with TOC in the biosolids-amended soils (both $p > 0.05$, Fe: $r = -0.77$; Al: $r = -0.40$). TOC and Fe from 0 to 10 cm in the control at the Solano site were moderately correlated ($r = 0.41$, $p = 0.73$), with similar correlation found from 75 to 100 cm ($r = 0.228$, $p = 0.924$), weaker correlations observed at the 30–50 cm depth ($r = 0.02$, $p = 0.98$). Correlations between TOC and Al in the Solano control soils
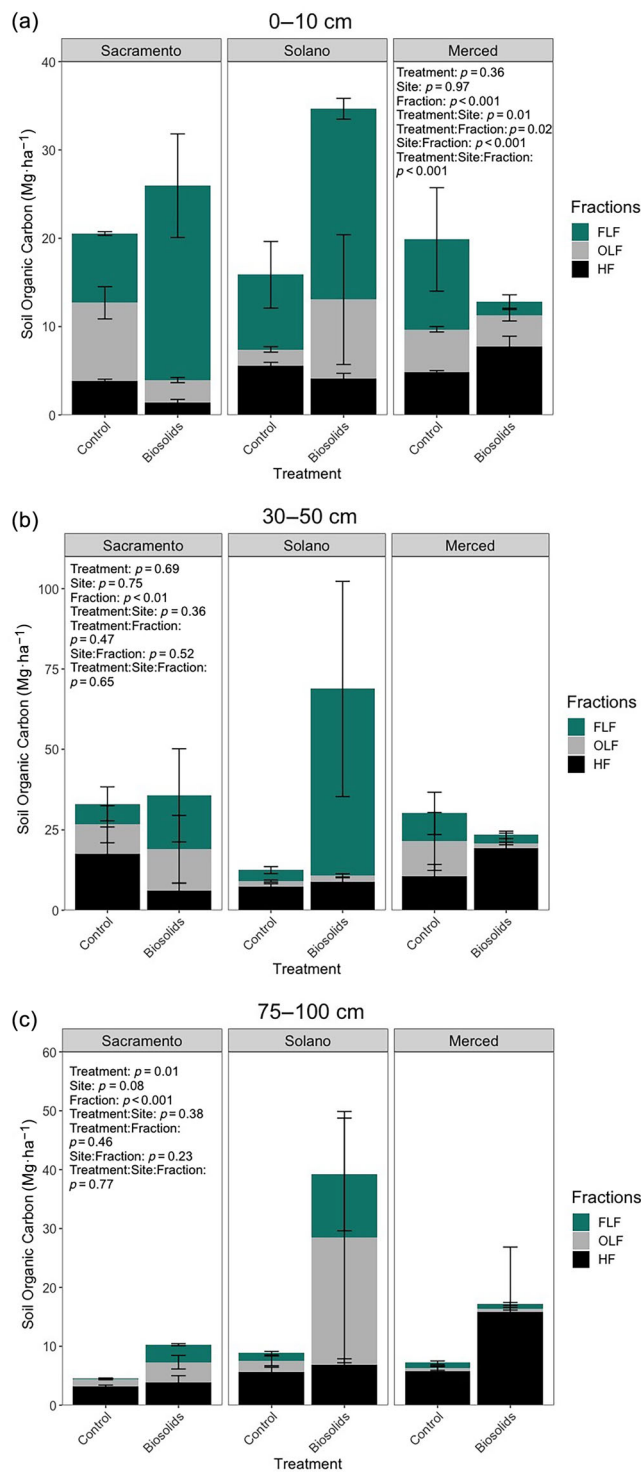
FIGURE 1 The component of density fractionations to soil organic carbon along three soil depths ([a] 0–10 cm, [b] 30–50 cm, and [c] 75–100 cm) in three different sites. Error bars show standard errors. FLF, free-light fraction; OLF, occluded-light fraction; HF, heavy fraction.
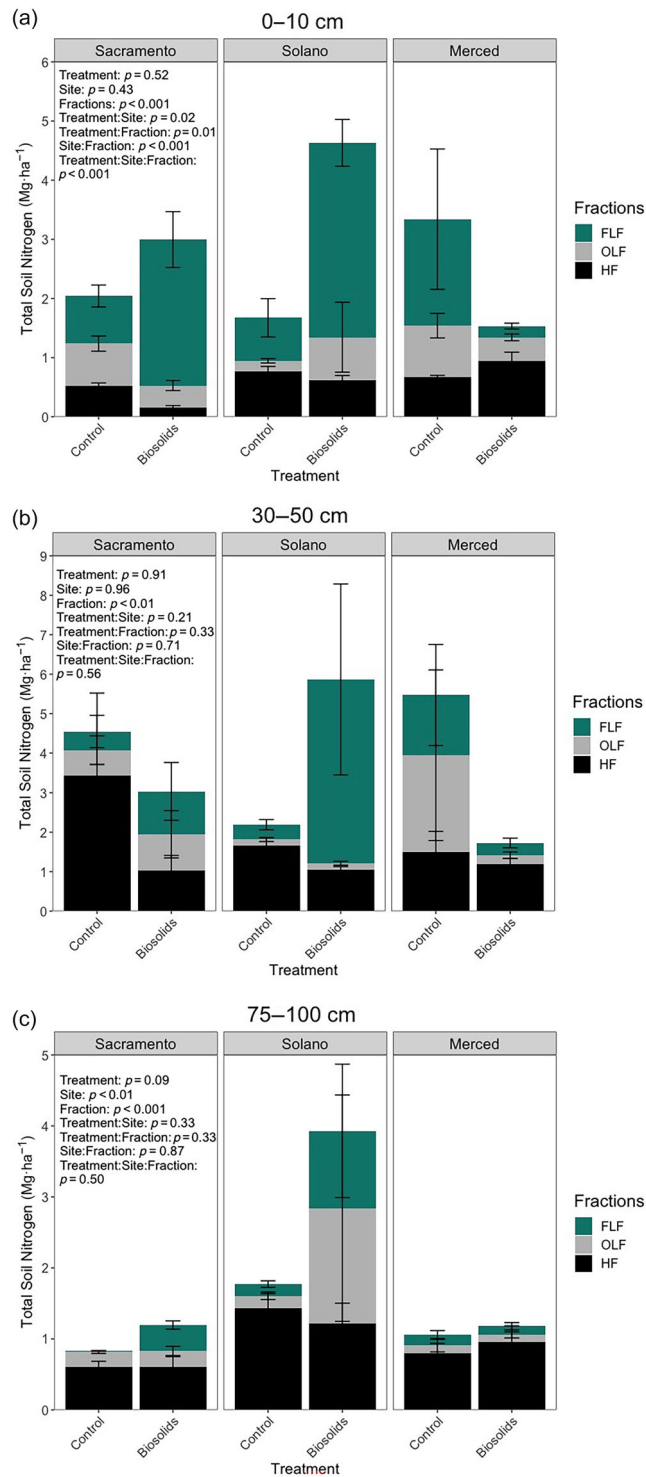
FIGURE 2 The contribution of three soil fractions to total soil N content: (a) 0–10 cm, (b) 30–50 cm, and (c) 75–100 cm. Error bars are standard errors. FLF, free-light fraction; OLF, occluded-light fraction; HF, heavy fraction.
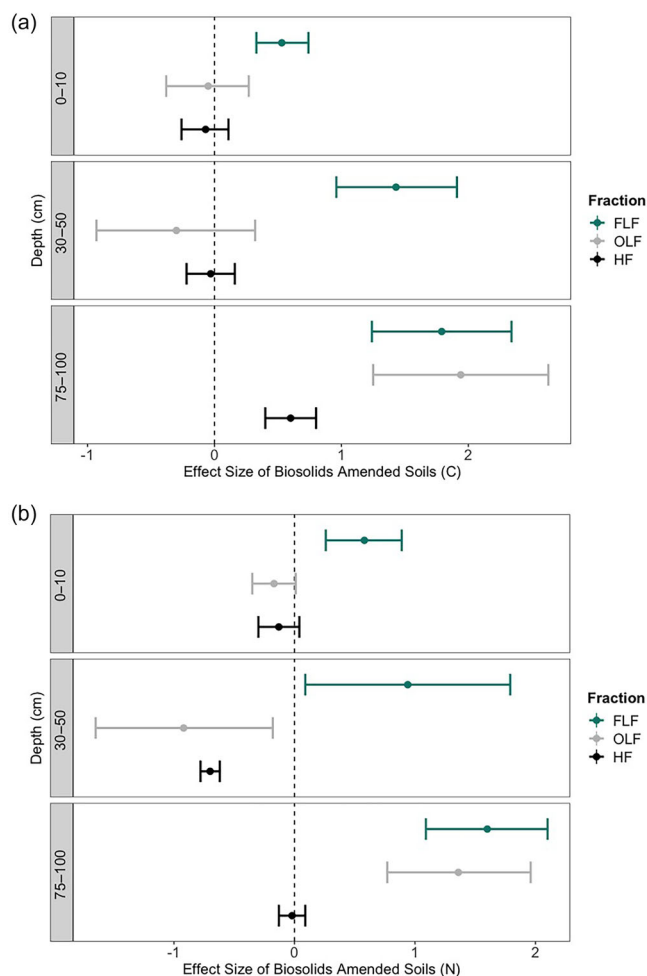
FIGURE 3 Plot showing effect size of (a) C and (b) N across all sites. Error bars are showing the variance. FLF, free-light fraction; OLF, occluded-light fraction; HF, heavy fraction.

show a moderately negative correlation from 0 to 10 cm ($r = -0.70$, $p = 0.52$) and an increasingly positive correlation along depths (30–50 cm: $r = 0.05$; 75–100 cm: $r = 0.397$; both $p > 0.05$). TOC and Fe are weakly correlated from 0 to 10 cm ($r = 0.1$), negatively correlated in the biosolids-amended soils at the Solano site from 30 to 50 cm ($r = -0.996$; $p = 0.052$), and weakly correlated again from 75 to 100 cm ($r = 0.228$, $p = 0.92$). The TOC and Al correlations in the biosolids-amended soils at the Solano site also had negative correlations from 0 to 10 cm ($r = -0.05$, $p = 0.96$) and from 30 to 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 and Fe and Al for both treatments along depths, except for the cor-
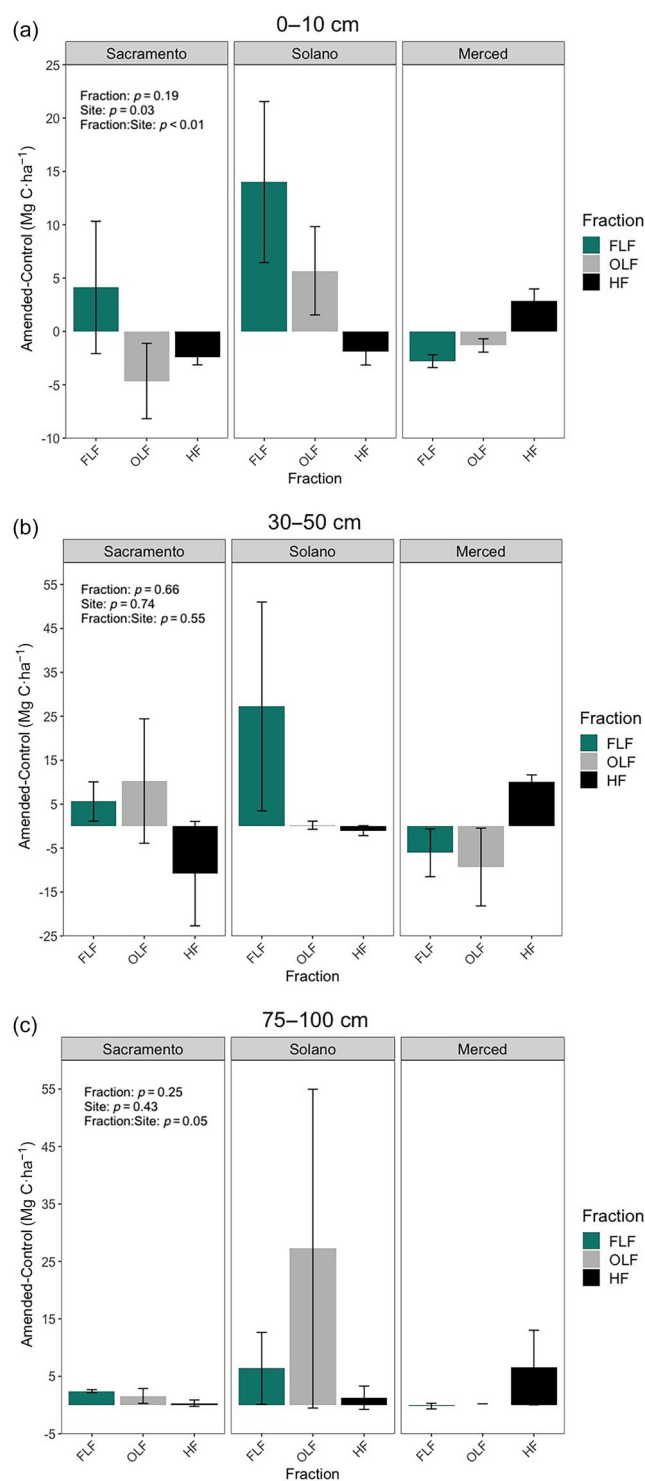

FIGURE 4 The treatment effect of biosolids application on total soil organic carbon storage for each soil fractions from the three depths: (a) 0–10 cm, (b) 30–50 cm, and (c) 75–100 cm. Error bars are standard errors. FLF, free-light fraction; OLF, occluded-light fraction; HF, heavy fraction.
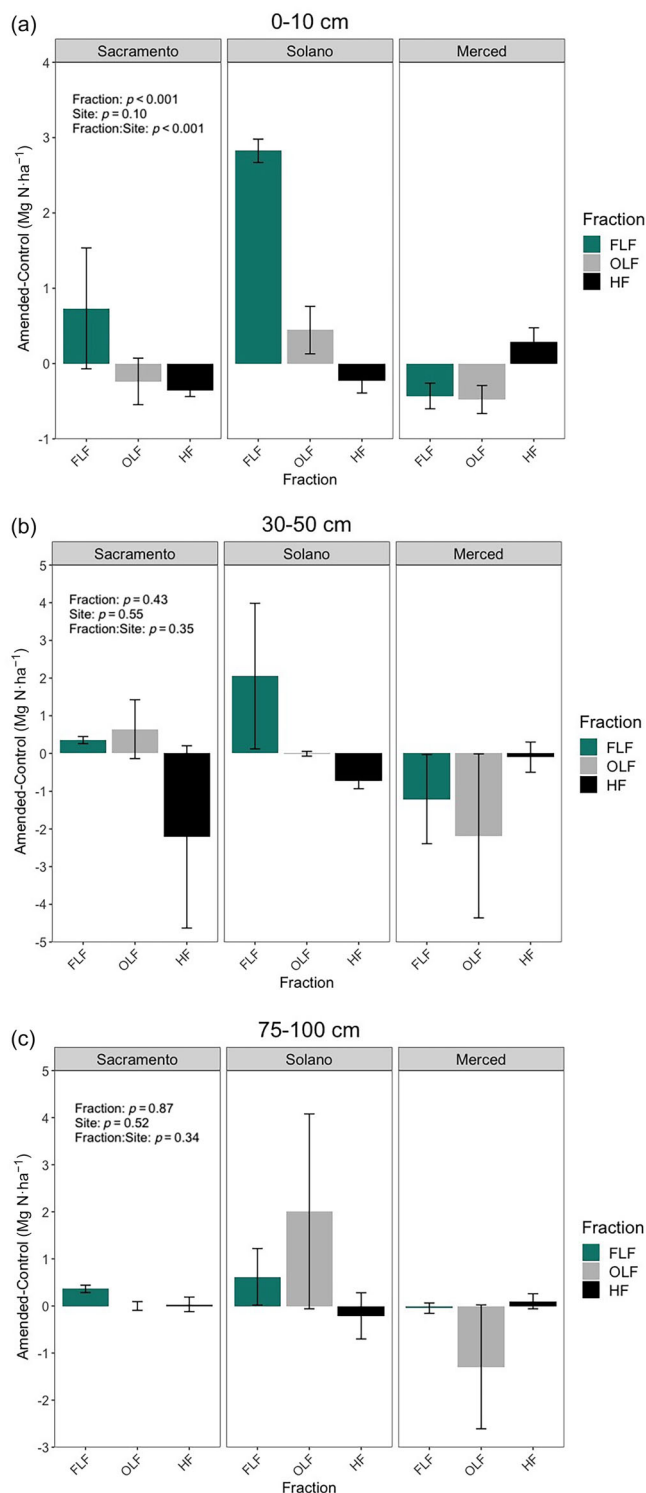
FIGURE 5 Treatment effect on total soil N content for each soil fraction across depths: (a) 0–10 cm, (b) 30–50 cm, and (c) 75–100 cm. FLF, free-light fraction; OLF, occluded-light fraction; HF, heavy fraction.

relation between TOC and Al in the biosolids-amended soil from 0 to 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 to 10 cm; TOC:Al from 30 to 50 cm; TOC:Fe/Al from 75 to 100 cm; biosolids: TOC:Al from 30 to 50 cm; TOC:Fe from 75 to 100 cm).

3.4 | Correlations between SOC and Ca^{2+} in Merced

Stronger correlations between Ca^{2+} and SOC concentration were observed from the control HF compared to the long-term amended soils across all depths (Figure 6). Correlations were strongest at the 0–10 cm depth for each treatment compared to other depths where the control had a correlation coefficient of 0.94 ($p = 0.22$) and the biosolids amended had a correlation coefficient of 0.03 ($p = 0.98$). The 30–50 cm depth 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 ($r = -0.88$, $p = 0.31$), and the control showed a minimal correlation between Ca^{2+} and SOC concentration, though not negative ($r = 0.31$, $p = 0.80$).

4 | DISCUSSION

4.1 | Effects of long-term biosolids applications on soil density fractionations

Fractionation techniques are a valuable tool that can provide indications of 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). Our study found that relative proportions of C and N found in each fraction differed along depths, sites, and treatments. Effect sizes across our study show the impact of how biosolids application and measuring deep soil C can provide a better assessment of the C sequestration potential of a soil. The effect sizes of both C and N in the FLF increased in surface soils and strengthened in deeper soil layers, even though biosolids are only incorporated to 30 cm. Across all fractions, the effect sizes increased in the 75–100 cm depth, indicating a stronger relationship among depth, C, and N (Figure 3a,b). As SOC and total N move to subsoils, the turnover rate tends to decrease, thus remaining for longer periods of time (Hicks Pries et al.,
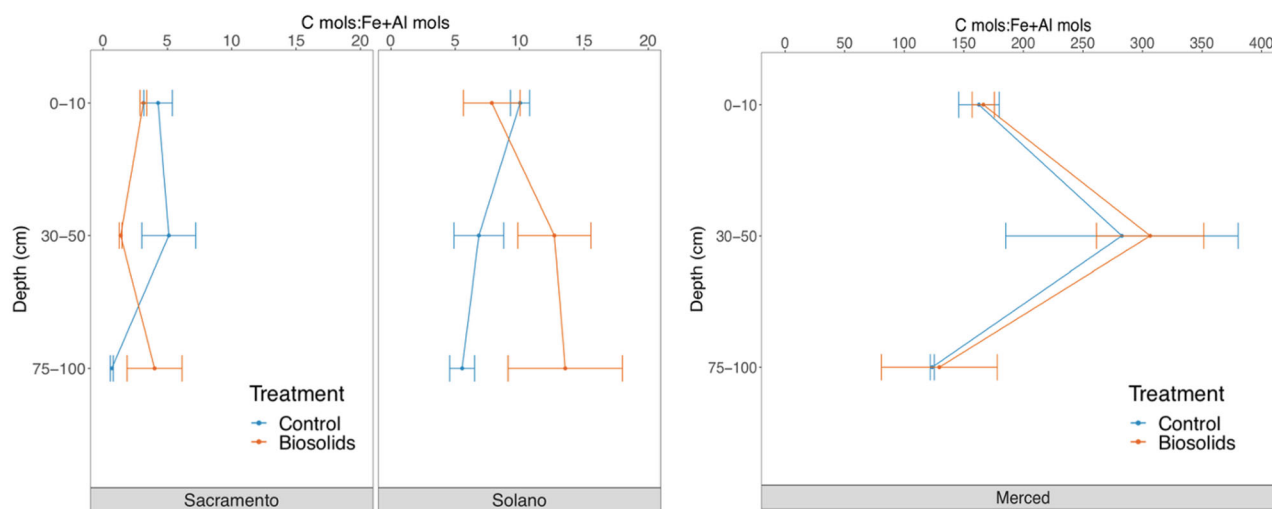
FIGURE 6 Moles of C per moles of M (Fe + Al).

2017; Jackson et al., 2017; Shi et al., 2020; Tautges et al., 2019). Subsoils also typically contain higher concentrations of clays and organomineral complexes that can contribute to C stabilization mechanisms (Kögel-Knabner et al., 2008).

As expected, the TOC and total N content associated with the FLF from 0 to 10 and 30 to 50 cm increased in the biosolids-amended soil, but only in the Sacramento and Solano sites. The higher proportions of FLF in the amended soils from the Sacramento and Solano sites are likely from two sources. The first likely source is the C and N content from the biosolids that persisted over time. One 547-day incubation study looked at the chemical composition of organic amendments, including biosolids, and how the decomposition rates were influenced by this factor (Baldock et al., 2021). The study found that biosolids tended to emit less CO_2 and that most of the total C from biosolids was allocated in slow mineralizing pools. The second likely source of additional FLF C and N is from above- and belowground plant litter through the promotion of plant production (Ryals & Silver, 2013). Several studies have found that biosolids increase SOC and plant production after a one-time application (Bolan et al., 2013; 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 active vertical transport into deeper soil layers showing increased input from plant roots (Silva et al., 2015).

Deeper soil depths had higher proportions of C and N in the FLF and OLF only at the Sacramento and Solano sites. The C and N present in the control soils at the Sacramento and Solano sites had organic C and total N associated with HF at relatively high proportions. Similarly, many cases have

shown that the N associated with the HF in the control soils was higher in proportion than in the amended soil. The higher proportions of C and N associated with 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; Lin et al., 2022; Silva et al., 2015).

The Merced site did not experience an increase in C and N associated with FLF from 0 to 10 cm or from 30 to 50 cm. Similarly, N did not increase from 0 to 10 cm, but the 30–50 cm depth remained unchanged. This may be attributed to a coarser soil texture at the Merced site that can facilitate the movement of SOC to deeper soil. When comparing the unamended control and the biosolids-amended soils at Merced, a shift in C and N associated with fractions differs. Unamended controls had higher proportions of C and N associated with the FLF than the amended soil where higher proportions of C and N were associated with the HF (Tables S4 and S5). This shift from FLF to HF suggests that the C and N content from 0 to 10 and 30 to 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 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 the higher amounts of C and N associated with HF through practices such as flood irrigation and tillage. The Merced site has a coarse texture, experiences the fewest frequency of biosolids application, engages in an alfalfa–corn rotation with its soils, resulting in tillage, and undergoes flood irrigation. 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 previously protected in aggregates (Stewart et al., 2012).

An important management process that affects C stabilization is the use of tillage to incorporate biosolids. All biosolids transects were tilled from 0 to 30 cm, and the control transect at the Merced site was tilled during the corn or alfalfa harvesting. Additionally, the frequency of tillage and biosolids practices varies across sites (Sacramento: yearly; Solano: every 3 years; and Merced: every 5 years; Table S1). The Sacramento site received biosolids application yearly, but also underwent tillage practices that may have caused C losses. The total C content between the biosolids and control did not significantly change from 0 to 10 cm, but more C was associated with the FLF, showing that the application of biosolids is introducing more labile C but potentially losing more stable fractions at the Sacramento site (Figure 1a). From 0 to 10 cm at the Solano site, there was a significant increase in C associated with the FLF and OLF, suggesting that the 3-year span between application and tillage of biosolids was enough time to allow the movement of C to deeper soil layers, as well as the promotion of plant growth (Figure 1). Some studies have shown that tillage may promote the accumulation of SOC below the plow layer compared to no-till practices, which have been shown to accumulate in surface soils (Angers & Eriksen-Hamel, 2008; Six et al., 2002; West & Post, 2002). Management practices influence C input, C distribution, and C decomposition processes across the soil profile (Luo et al., 2010; Six et al., 1999). The importance of investigating below-surface soil has been argued in order to understand the extent of management-induced changes to soil C stock (Berhe et al., 2008; Lal, 2009; Soong et al., 2021; Tautges et al., 2019; Villa & Ryals, 2021).

4.2 | Organomineral complexes are dependent on pH

Complexation of SOC to Al and Fe was more strongly correlated at the Sacramento and Solano sites than at the Merced site (Figure 7). We calculated the C:M ratio (Fe+Al mols) to determine whether there is enough stock of metal ions in the soil for chelation to have a significant influence on C storage in soils across the three sites, treatments, and depths. Following Oades (1989), Masiello et al. (2004), and Berhe et al. (2012), we assumed each organic C functional group represents SOC C atoms associated with one negative charge. If every OC functional group was bound to a metal ion and metal ions existed in the +1-charge state, the molar C:M ratio would be 6 (Masiello et al., 2004). Any ratio above 6 would indicate that there is not enough stock for Al and Fe to bind to SOC. How-

ever, it can be assumed that not all SOC and metal ions will fall under these assumptions. Some OC can have many functional groups that can interact with one metal ion, and some metal ions exist as polycations (raising the C:M ratio; (Berhe et al., 2012; Masiello et al., 2004). Hence, the bonding of all OC to M falls within a ratio range between 2 and 10 (Oades, 1989).

At the Sacramento and Solano sites, the C:M ratio generally falls within the 2–10 range (Figure 6), except in the biosolids-amended soil at the Solano site from 30 to 50 and 75 to 100 cm, where ratios were higher than 10. This may be due to more C to deeper soil layers at the Solano site, thus indicating there are not enough Fe and Al ions to bind to excess C present in the soil. The Merced site has a very large C:M ratio, ranging from 100 to 350. One possible explanation for this finding is that the soil at the Merced site has a pH of 8.0, and 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., 2017, 2018). Both the Sacramento and Solano sites had a pH of 5.9 in the top 30 cm of soil, making these sites ideal candidates for estimating organomineral complexes. The alkalinity of the soil at the Merced site could 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 30 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 C and N in these plots may 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 into the soil (Mikutta et al., 2007; Tian et al., 2013). In certain conditions, the introduction of metal oxides found in biosolids and SOC can become closely related due to organic matter carrying negative charges and metals having positive charges (Chiu & 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 than Fe or Al in the soil. Since the composition of the biosolids applied was similar across sites, it could be deduced that due to the annual high frequency of biosolids application, there was a higher presence of Fe and Al in the Sacramento soils compared to the Solano and Merced sites.
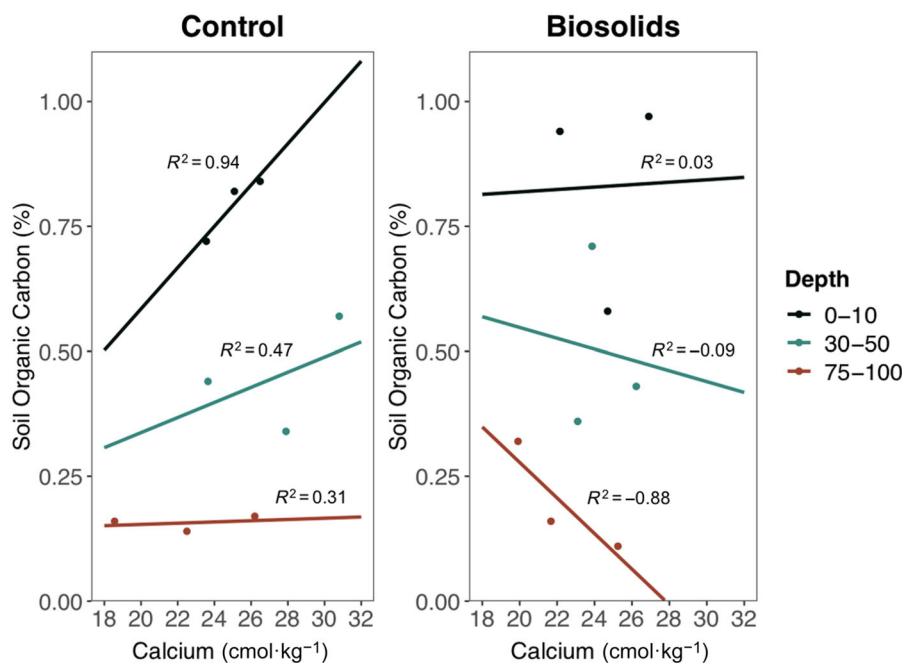
FIGURE 7 Relationship between soil organic carbon (%) and Ca^{2+} in the biosolids-amended soil and control at the Merced site using a Pearson correlation test.

4.3 | 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 (Solly et al., 2020; Weil & Brady, 2017). At the Merced site, soils had a pH of 8 from 0 to 100 cm and a high percentage of SIC (~22% of total C) in the form of calcium carbonate ($CaCO_3$) (Villa & 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., 2018, 2021; 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 with SOC chemically or physically. However, we found the opposite to be true (Figure 7). In fact, there is an ascending negative correlation moving down the soil profile in the amended soil compared to the control, where there is a weaker positive correlation moving down in depth. SOM has a high abundance of negatively charged sites that can improve cation exchange capacity (CEC), thus increasing soil C content in soils by reacting as a surface area in the soil where SOC may be adsorbed (Solly et al., 2020). Some studies have found that the application of organic matter amendments increases SOC and CEC

(Diacono & Montemurro, 2010). However, CEC is strongly associated with the available surface area of the soil (Farrar & Coleman, 1967; Solly et al., 2020), effectively influencing the ability of SOC to adsorb to reactive soil surfaces (Solly et al., 2020). One study found no significant increase of CEC in a coarse-textured almond orchard after the 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 to 100 cm, and the percentage of sand ranged from 53% (0–10 cm) to 73% (Villa & 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 in this study had a concentration of 35 ± 19 g·kg^{-1} (Villa & 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, it has a larger hydration shell than Ca^{2+} (Solly et al., 2020).

4.4 | Study limitations

While a strength of this study is the evaluation of long-term (20 years) biosolids application on soil C and N fractions, it is important to acknowledge the limitations of this approach. One limitation is the variability in site conditions, including

biosolids application rate and frequency, soil composition, and crop type, and site was often a significant factor influencing the results. To reduce the uncertainty associated with the long-term records of biosolids application, we used site-specific records of biosolids application rate and composition. All sites had detailed documentation on the Class B biosolids applied at all three sites, the frequency and amount applied, where it was applied, and the nutrient composition of the biosolids, due to reporting requirements. Unamended controls provided a point of comparison between soils amended with biosolids and how C dynamics change with the application of biosolids. Aside from biosolids application, all other management practices at the Merced site were held constant across treatment and control. However, at the grassland sites (Solano and Sacramento), biosolids were tilled in, while control areas were not tilled, leaving tillage management a confounding factor at these sites. These are challenges that are common when conducting research on working lands. Therefore, it is essential to continue studying C dynamics in different agroecological conditions to determine optimal agricultural contexts for biosolids reuse.

5 | CONCLUSIONS

Our study assesses the mechanisms that influence deep soil C sequestration in three managed soils that have had biosolids application for 20 years. Density fractionations demonstrated that amended soils had more C and N associated with the microbially accessible, free-floating fraction in surface and deep soils at the Sacramento and Solano sites. This was likely due 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 with HF in amended soils relative to the control, suggesting that the chemical composition of the C and N from biosolids application is changing. Organo-complexes were a stabilization mechanism in the Sacramento and Solano sites, indicating that the C associated with these metal ions will remain stabilized for long periods of time. Negative correlations between SOC and Ca^{2+} along depths relative to the control imply 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 in deep soils where it can persist for long periods of time across different agroecological contexts. We demonstrated the importance of accounting for deep soil C when assessing the climate change mitigation potential of managed soils.

AUTHOR CONTRIBUTIONS
Yocelyn Villa: Conceptualization; data curation; formal analysis; investigation; methodology; software; validation; visualization; writing—original draft; writing—review and editing.
Eddie Phosalath: Data curation; investigation; writing—review and editing. **Rebecca Ryals**: Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing—review and editing.

CONFLICT OF INTEREST STATEMENT
The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
Data generated for this study can be found at https://doi.org/10.6071/M33X22. Any other information required is available from the corresponding author on reasonable request.

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