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Authors

Ryals, R
Kaiser, M
Torn, MS
[et al.](#)

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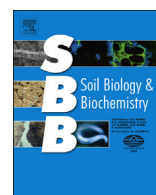
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Impacts of organic matter amendments on carbon and nitrogen dynamics in grassland soils



Rebecca Ryals ^{a,*,1}, Michael Kaiser ^{b,2}, Margaret S. Torn ^c, Asmeret Asefaw Berhe ^b, Whendee L. Silver ^a

^a University of California, Berkeley, Department of Environmental Science, Policy, and Management, 137 Mulford Hall #3114, Berkeley, CA 94720, USA

^b University of California, Merced, School of Natural Sciences, 4225 N Hospital Rd, Atwater, CA 95301, USA

^c Lawrence Berkeley National Laboratory, Earth Sciences Division, 1 Cyclotron Rd, Berkeley, CA 94720, USA

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ABSTRACT

Organic matter amendments have been proposed as a means to enhance soil carbon (C) stocks on degraded soils. However, only few data exist on rates of soil C sequestration or the fate of added C in grassland soils, which are generally thought to have high C storage potential. We measured changes in the amount of C and nitrogen (N) in soils and in the composition of soil organic matter (SOM) following a single application of composted organic matter in two annual grasslands from different bioclimatic zones (coastal and inland valley). There was a significant increase in bulk soil organic C content at the valley grassland, and a similar but non-significant trend at the coastal grassland. Physical fractionation of soil three years after organic matter amendment revealed increases in C and N in the free- and occluded light fractions in both the valley and coastal grasslands. Amendments resulted in a greater relative increase in the N stored in light soil fractions compared to C, leading to lower C:N ratios. Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectroscopy showed an increase in the ratio of carboxyl and carbonyl functional groups to aliphatic methyl and methylene groups in the free- and occluded light fractions. These data show that the organic matter amendment was incorporated in the free light and occluded light fractions over three years. Our results indicate that a single application of compost to grassland soils can increase soil C and N storage in labile and physically protected pools over relatively short time periods and contribute to climate change mitigation.

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1. Introduction

Soil organic matter (SOM) plays an important role in ecosystems by retaining and supplying plant nutrients, improving soil aggregation, reducing soil erosion, and enhancing water holding capacity (Tisdall and Oades, 1982; Brady and Weil, 2002). Grassland soils are generally thought to be rich in organic matter, but poor management, vegetation shifts, and changes in climate have decreased SOM stocks in many of the world's grasslands (Asner et al., 2004; Bai et al., 2008). The large global extent of grasslands with

depleted organic matter stocks has focused attention on the management of these ecosystems for C sequestration to help mitigate climate change (Asner et al., 2004; Bai et al., 2008; Conant et al., 2011; Follett, 2001; Lipper et al., 2010; Morgan et al., 2010). Management-induced soil C sequestration has been studied in agricultural systems in the context of conservation tillage, cropping, organic and synthetic fertilization, and residue incorporation (Paustian et al., 1997; Lal, 2002; Kaiser et al., 2007; Kong et al., 2009; Lugato et al., 2010). In contrast, information regarding the impact of grassland management on soil C sequestration is much more limited (Conant et al., 2001; Follett, 2001; Lal, 2002; Derner and Schuman, 2007).

Organic matter amendments have been proposed as a means to increase C storage in soils (Cabrera et al., 2009; Powlson et al., 2012); this can occur directly from the C inputs in the amendment and indirectly from increased plant production (Eghball and Power, 1999; Ryals and Silver, 2013). Organic matter amendments to soils have been related to enhanced soil water holding capacity (Gagnon et al., 1998; Zebarth et al., 1999; Pandey and Shukla, 2006),

* Corresponding author. Present address: Brown University, Environmental Change Initiative, MacMillan Hall Box #1951, Providence, RI 02906, USA. Tel.: +1 401 863 2799.

E-mail addresses: rebecca_ryals@brown.edu, rebeccaryals@gmail.com (R. Ryals).

¹ Now: Brown University, Environmental Change Initiative, 167 Thayer St, Providence, RI 02912, USA.

² Now: University of Kassel, Department of Environmental Chemistry, Nordbahnhofstr. 1a, 37213 Witzenhausen, Germany.

decreased bulk density (Lynch et al., 2005), and improved soil fertility (Mader et al., 2002). Amendments may also increase organic nitrogen (N) in soils, which could act as a slow release fertilizer enhancing net primary productivity (Ryals and Silver, 2013). Furthermore, organic matter amendments could provide opportunities for greenhouse gas offsets if materials are diverted from high emissions sources, such as food waste from landfills (Powelson et al., 2012; DeLonge et al., 2013).

However, the fate of organic matter amendments in grassland soils remains unclear. Most organic matter amendments are applied to the soil surface. The proportion of this material that is incorporated and retained in soils over time is unknown, but is likely a function of the chemical composition of the material added and the specific soil and environmental conditions of the site. Several mechanisms can act to stabilize added C and N including physical protection via soil aggregation by, for example, microbial production of binding agents in the course of organic matter decomposition (Golchin et al., 1994; Six et al., 1998; Gulde et al., 2008). Detecting and interpreting changes to soil C and N pools can be difficult, particularly in ecosystems with relatively large standing stocks of bulk soil C and N. If organic matter that is applied to the surface is not sorted or separated when soils are sampled and analyzed, increases in soil C and N stocks may be greatly exaggerated.

Soil physical fractionation techniques can improve our ability to detect management-induced changes in pools of C and N, and have been used widely in agricultural and forested ecosystems (Chivenge et al., 2007; He et al., 2008; Powelson et al., 2012). Fractionation provides an indication of the physical location of C and N in the soil matrix (Sollins et al., 1996). Turnover of SOM is governed, in part, by the accessibility of organic substrates to decomposers (Dungait et al., 2012), and also by the chemical quality of the material (Jastrow et al., 2007; Conant et al., 2011). Assessments of SOM chemical characteristics are commonly used to infer its potential reactivity (Kögel-Knabner et al., 2008). By combining physical fractionation and chemical characterization, it is possible to identify fractions with different SOM stabilization potentials (Sohi et al., 2001; Poirier et al., 2005) to assess their relevance for long-term soil C and N storage.

In this study, we investigated the effects of an organic matter amendment (compost) over a three-year period on C and N storage in two grassland soils. We used a combination of field and laboratory approaches to determine the fate of the amendment over time. We hypothesized that a single application of composted organic matter would promote soil C and N sequestration primarily through an increase in the free light fraction of the SOM pool over the short-term (3 y), that N storage would be lower relative to C in soil fractions due to losses during mineralization, and that sequestered C would be dominantly composed of the amendment material. We combined measurements of bulk soil C and N pools with physical fractionation procedures and Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectroscopy to track the fate of the added compost in the two grassland soils.

2. Material and methods

2.1. Study sites and experimental design

The study was conducted in valley grasslands at the Sierra Foothill Research and Extension Center (SFREC) in Browns Valley, CA (39.24 °N, 121.30 °W) and coast range grasslands (hereafter referred to as “coastal”) in Nicasio, CA (38.06 °N, 122.71 °W). Valley and coastal grasslands represent the two dominant grassland types in California (Kuchler, 1964; Brown et al., 2004): Valley grasslands occupy the hot, dry interior regions of the Central Valley and Sierra Nevada foothills. Coastal grasslands occur adjacent to the coast and

experience milder summertime temperatures and greater winter rainfall (Jackson and Bartolome, 2002). At the valley grassland site, annual precipitation averages 730 mm/y (22 y mean), and mean air temperatures range from 2 °C in January to 35 °C in July. Soils are derived from Mesozoic and Franciscan volcanic rock and classified as in the Auburn–Sobrante complex in the Xerochrepts and Haploeralfs great groups (Beaudette and O’Geen, 2009). At the coastal grassland site, annual precipitation averages 950 mm/y (37 y mean), and mean air temperatures range from 6 °C in January to 20 °C in July. Soils are derived from Franciscan mélange and classified as the Tocaloma–Saurin–Bonnydoon series in the Haploxerolls and Argixerolls great groups (Beaudette and O’Geen, 2009). Both grassland types are dominated by non-native annual grasses (e.g. *Avena barbata*, *Festuca perennis*), with native perennial grass species (e.g. *Stipa pulchra*, *Danthonia californica*) also present at the coastal grassland.

Paired plots (25 m × 60 m) were established in three watersheds at each site in October 2008. Treatments consisted of a one-time organic matter amendment and a non-amended control. An organic-rich soil amendment was added as a single application of commercially available composted dry organic green waste (Feather River Organics, Marysville, CA) with a N concentration of 1.87% and a C:N ratio of 11. A thin surface dressing approximately 1.3 cm thick (equivalent to 7 kg/m², 1.42 kg C/m², and 129 g total N/m²) was applied in December 2008.

2.2. Organic matter amendment characteristics

Subsamples of the amendment were collected from multiple locations in the compost pile, dried at 65 °C, finely ground, and analyzed for C and N concentration on a Carlo Erba Elemental Analyzer (Lakewood, NJ). Three 250 g subsamples of compost from the same feedstock were used to determine particle size class components. These subsamples were separated into the size classes <1, 1–2, 2–4.75, and >4.75 mm through sequential sieving. Each size class was weighed, finely ground, and analyzed for C and N concentration in duplicate on a Carlo Erba Elantech elemental analyzer (Lakewood, NJ) using atropine as a standard.

2.3. Soil texture, pH, and bulk density

Soil texture was determined using the hydrometer method adapted from Gee and Bauder (1986). We sampled soils along three transects in each plot (0–10 cm depth) using a 6 cm diameter corer; three samples were collected and composited per transect to yield 3 sample replicates per plot and 9 samples replicates per treatment at each site. Soils were air dried, and rocks were removed with a 2 mm sieve prior to texture analyses. Soil pH was measured in a ratio of 1:2 soil to water (McLean, 1982).

Soil bulk density was measured to 100 cm depth in 10 cm depth increments in one pit per plot. We carefully excavated 10 cm diameter cores approximately 5 cm back from the face of the pit. All bulk density measurements were corrected for rock volume and mass. Upon extraction from cores, dry rock masses and volumes were measured and subtracted from total bulk density calculations (Robertson et al., 1999).

2.4. Total soil organic carbon and nitrogen

Soils were collected prior to compost application and at end of each water year (WY; typically May or June) for three years using a 7 cm diameter corer ($n = 9$ per depth per plot). Sample depths were 0–10, 10–30, 30–50, and 50–100 cm at the coastal grassland and 0–10, 10–30, and 30–50 cm at the valley grassland where soils were shallower. Roots and identifiable compost litter were

removed by hand sorting in the lab. Soils were then air-dried and pulverized with a ball grinder (SPEX Sample Prep Mixer Mill 8000D, Metuchen, NJ). No carbonates were present based on a lack of effervesce upon addition of 4 N HCl to soil. Carbon and N concentrations were measured in duplicate on a CE Elantech elemental analyzer (Lakewood, NJ) as above. Carbon and N concentrations were converted to content using bulk density values specific to each plot and depth.

2.5. Density fractionation

Density fractionation methods can be used to as an early indicator of management-induced changes to soil C and N storage, and are generally more sensitive to changes than measurements of bulk soil C and N reservoirs over short timescales. Bulk soil C and N were partitioned into three fractions using a physical density fractionation procedure (Swanston et al., 2005; Marín-Spiotta et al., 2008; Cusack et al., 2011). This method defines fractions as the low-density free light fraction (FLF), the physically protected occluded light fraction (OLF), and the mineral-associated heavy fraction (HF). This procedure separates organic matter according to its physical location in the soil and the fractions typically have different turnover times (Swanston et al., 2005; Marín-Spiotta et al., 2008). The FLF chemistry closely resembles plant material and is particulate organic matter free-floating in the soil matrix. It often has the fastest turnover time and is generally the most easily accessible to decomposer microorganisms and their extracellular enzymes. The OLF consists of a pool of C and N that is physically protected in soil aggregates and therefore less accessible to decomposers, and generally has longer residence time than the FLF. The heavy fraction consists of mineral-associated C and N and typically has the longest residence time in soils.

Soils for density fractionation (0–10 cm depth) were collected at the end of WY 3 in 2011 from all treatment plots at both sites using a 7 cm corer. Three cores were composited along three transects within each of the twelve plots, for a total of 36 samples ($n = 3$ sample replicates per 3 treatment or control replicates per site). Methods for soil fractionation were adapted from Swanston et al. (2005). Rocks were removed by passing soil through a 2 mm sieve, and roots were extracted by hand. Approximately 20 g was weighed into centrifuge tubes, and 75 mL of sodium polytungstate (NaPT, $\text{Na}_6 [\text{H}_2\text{W}_{12}\text{O}_{40}]$ TC-Tungsten Compounds, Bavaria, Germany) dissolved to a density of 1.85 g cm^{-3} was added. Samples were gently shaken and centrifuged before aspirating off the light density, floating FLF. The remaining portion of sample was then agitated to disrupt microaggregates with a benchtop mixer (G3U05R, Lightning, New York, NY) at 1700 rpm for 1 min and sonicated in an ice bath for 3 min at 70% pulse (Branson 450 Sonifier, Danbury, CT). The liberated OLF was then collected via aspiration after centrifugation. The remaining dense HF was rinsed with deionized water and centrifuged until all NaPT was removed. The NaPT was removed from the FLF and OLF by rinsing with deionized water through a $0.8 \mu\text{m}$ polycarbonate membrane filter (Whatman Nuclepore Track Etch Membrane). The light fractions and HF were dried in tins at $65 \text{ }^\circ\text{C}$ and $105 \text{ }^\circ\text{C}$, respectively, until weights stabilized. All fractions were ground using a ball mill and analyzed for C and N concentrations as described above. Average mass recovery was $98.4 \pm 0.2\%$.

2.6. Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectroscopic analyses

Soil fractions and bulk compost were analyzed for chemical characteristics via Diffuse Reflectance Infrared Fourier Transform spectroscopy (DRIFT) (Nguyen et al., 1991). Spectra were recorded

with a Fourier transform infrared (FTIR) spectrophotometer (Bruker IFS 66v/S, Ettlingen, Germany) equipped with a Praying Mantis DRIFT apparatus (Harrick Scientific Corporation, Ossining, NY). Samples were prepared by homogenizing a 4 mg sample and 40 mg spectroscopy-grade KBr (Aldrich Chemical Co., Milwaukee, WI) with a mortar and pestle. Finely ground KBr powder was used as a background reference. Absorption spectra were obtained in the mid-IR range of wavenumbers between 4000 and 400 cm^{-1} averaged over 32 scans at 4 cm^{-1} resolution and converted to Kubelka–Munk units. Peaks of DRIFT spectra represent overlapping bands of compounds (Nguyen et al., 1991).

The peak heights of four absorption maxima (i.e., peaks) were recorded from each spectrum (Table 1). Peaks at wavenumbers 2910–2930 and 2853 cm^{-1} are derived from aliphatic methyl and methylene groups and were grouped into band A. Peaks at wavenumbers 1653 and 1400 cm^{-1} are derived from oxygen containing carboxyl and carbonyl groups and were grouped into band B. The peak at 1400 cm^{-1} may also indicate carbonates. However, soils tested negative for carbonates following the addition of HCl. Thus, these band regions are not affected by mineral interference, and ash spectra subtraction was not required (Günzler and Böck, 1990; Ellerbrock et al., 2005). Tangential baseline corrections were made when applicable (Smidt et al., 2002). Peak heights covered under band B relative to those covered under band A (i.e., B:A ratio) was used as an index of the degree of organic matter decomposition for a given sample (Kaiser et al., 2007). The B:A ratios were further normalized to the organic C concentration of the sample (i.e., B:A/% C, referred to hereafter as B:A*) (Djukic et al., 2010). Each field replicate of each soil fraction was analyzed individually. Mean spectra and standard errors were computed at the plot scale.

2.7. Statistical analyses

Treatment differences in bulk soil C and N concentrations and pools at each grassland site were determined using analysis of variance (ANOVA) on plot averages. Analyses included a blocking effect and were performed separately for valley and coastal sites. Repeated measures ANOVAs were employed to explore treatment effects through time with block, treatment, time, and interactions as model effect factors. ANOVAs were used to identify statistically significant treatment effects on C and N in the density fraction mass, concentration and content. DRIFT peak heights and B:A* were analyzed using ANOVA to detect potential differences among C and N fractions, treatments, and grassland sites. Correlations among total soil C and N content, soil fractions C and N content, chemical composition of soil fractions, and soil texture were explored using ordinary least squares multiple linear regressions.

Table 1
Selected infrared bands for soil fraction DRIFT spectra.

Wavenumber (cm^{-1})	Vibration	Functional group or compound
2910–2930	Asymmetric C–H stretching	Aliphatic methylene groups ^{a,b,c,d}
2853	Symmetric C–H stretching	Aliphatic methylene groups ^{a,b,c}
1653	C=O stretching	Amid I, carboxylates ^e
	C=C stretching	Aromatic ring modes, alkenes ^{b,d,f}
1400 ^g	COO- stretching	Carboxylic acids ^b

^a Tatzber et al. (2007).

^b Smith (1999).

^c Smidt et al. (2002).

^d Simonetti et al. (2011).

^e Haberhauer et al. (1998).

^f Ouattmane et al. (2000).

^g This peak may also be indicative of carbonates. However, the lack of effervesence upon the addition of HCl to the soil suggests that carbonates are not present in these soils.

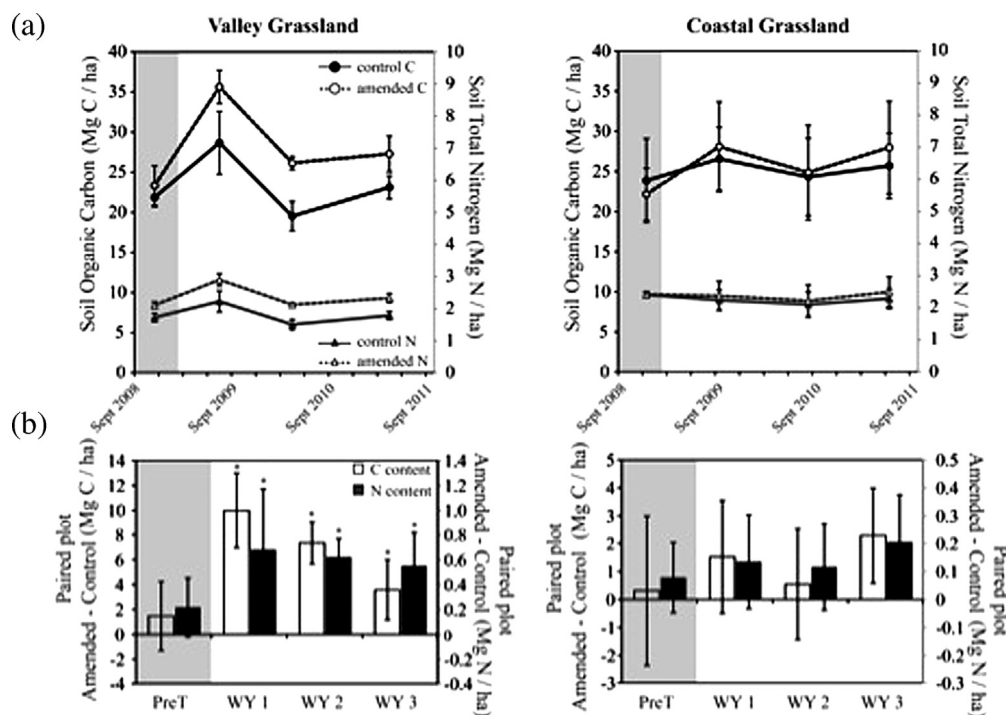


Fig. 1. (a) Total soil organic C and total N content to 10 cm depth prior to and three years following organic matter amendment at two grassland types. Circles with solid lines represent C and N content of controls soils, and circles with dashed lines represent those of amended soils. (b) Treatment effects are shown as paired plot differences between amended and control soils. Positive values indicate greater C or N in amended soils versus control soils. Soils were sampled annually, at the end of each water year (WY). Pre-treatment (PreT) data are shaded. * Indicates $p < 0.05$ for differences between paired plots.

Statistical tests were performed using JMP 7.0.2 (SAS Institute Inc.). Variables that were not normally distributed were log transformed to meet assumptions for ANOVA. Data are reported either as mean values or treatment differences followed by ± 1 standard error. Error propagation was used when applicable. Statistical significance was determined as $p < 0.05$ unless otherwise noted.

3. Results

3.1. Soil characteristics

Initial soil pH was 6.44 ± 0.05 and 5.92 ± 0.13 at the valley and coastal grassland, respectively ($p < 0.005$). There were no statistically significant differences in initial soil pH across blocks or plots within a site. Organic matter amendments increased pH slightly at the valley grassland ($p < 0.05$, Appendix Table 1).

The two grassland soils had similar soil texture, with no significant difference between treatments. Mean percentages of clay, silt, and sand were 16 ± 2 , 42 ± 1 , and $42 \pm 2\%$ at the valley grassland and 16 ± 2 , 40 ± 2 , and $43 \pm 4\%$ at the coastal grassland. At the coastal grassland, one block contained significantly more sand ($54 \pm 1\%$ versus $38 \pm 2\%$) and 54% less clay ($12 \pm 1\%$ versus $18 \pm 1\%$) than the other two blocks ($p < 0.0001$). Organic matter amendments did not significantly alter soil texture.

Soil bulk density ranged from 0.87 ± 0.05 to 1.27 ± 0.03 g/cm³ and increased with depth in both grassland sites ($p < 0.001$ at both sites; Appendix Table 1). Soils were shallower at the valley grassland, with bedrock around 50 cm depth versus >100 cm at the coastal grassland. Soil bulk density was significantly greater at the coastal grassland versus the valley grassland below 30 cm ($p < 0.05$). There were no statistically significant effects of amendments on bulk density.

3.2. Soil carbon and nitrogen

Soil C and N concentrations declined significantly with depth ($p < 0.0001$), and there were no significant differences across treatment plots at any depth increment prior to the compost application. By the end of the first water year, soil C and N concentrations increased significantly in the 0–10 cm depth at the valley grassland ($p < 0.05$ for % C and % N). There was a similar, albeit not statistically significant, trend at the coastal grassland. These patterns persisted; after 3 years there was a general positive effect of the treatment on bulk soil C and N concentrations following a single application of composted organic material, particularly in surface soils (Appendix Table 1). Soil C and N concentrations exhibited interannual variability, with stronger interannual variability occurring at the valley grassland.

Soil C and N content of surface (0–10 cm) soils were greater in amended versus control plots by the end of year one and remained elevated by the end of year three at the valley grassland (Fig. 1a). At the end of year three, the total soil C content of the valley site was 53 ± 2 Mg C/ha and 63 ± 5 Mg C/ha in 0–50 cm depth for control and amended plots, respectively. Total N in 0–50 cm depth was 3.17 ± 0.14 Mg N/ha for the control and 3.73 ± 0.23 Mg N/ha for the amended plots. The positive effect on soil C content was greatest in the first year following amendment application. The positive effect on N content was sustained through all three years following amendment (Fig. 1b). Similar trends occurred at the coastal site, although differences were not statistically significant. Changes in soil C and N contents were due primarily to differences in C and N concentrations as there were no significant treatment effects to bulk density at either site (Appendix Table 1).

3.3. Soil density fractions

Organic matter amendments significantly increased the C content of the FLF by 2.56 ± 1.13 Mg C/ha and 1.82 ± 0.99 Mg C/ha in the top 10 cm of soil at valley and coastal grasslands, respectively (Fig. 2a–b). The C content of the OLF at the coastal grassland increased by 1.29 ± 1.75 Mg C/ha ($p < 0.05$) with a significant block effect ($p < 0.001$), and the OLF C content at the valley grassland showed a similar but non-significant trend. The HF C content did not vary significantly by treatment. The HF had lower C concentrations than the FLF and OLF (approximately 1.5% versus 22% and 31%, respectively), but contained most of the soil C because it comprised >93% (valley) and >96% (coastal) of total soil mass (Table 3).

Nitrogen concentrations and content increased significantly in all soil fractions at the valley grassland and in the FLF and OLF at the coastal grassland (Fig. 2c–d). Amendments significantly decreased C:N ratios of surface soils from 19.2 ± 0.7 to 14.2 ± 0.2 in the FLF at the valley grassland, and from 18.8 ± 1.2 to 15.8 ± 0.9 in coastal grassland (Table 3). The C:N ratio of the OLF decreased significantly with amendments from 18.0 ± 0.8 to 15.4 ± 0.6 , and from 16.5 ± 0.5 to 14.6 ± 0.1 at the valley and coastal grassland, respectively. The C:N ratio of the HF did not differ between sites or treatments and was 9.6 ± 0.3 . There were significant correlations between C and N concentrations when all soil fractions were pooled ($r^2 = 0.98$, $p < 0.0001$ at both sites). This relationship was most pronounced in the HF ($r^2 = 0.66$ and 0.99 in the valley and coastal grassland sites, respectively; $p < 0.05$, $n = 6$ for each site) and OLF ($r^2 = 0.80$ and 0.61 in the valley and coastal grassland sites, respectively; $p < 0.05$, $n = 6$ for each site). The correlations between C and N concentrations in the FLF were not significant at either grassland.

Nearly two-thirds of all soil organic C was stored in the HF at the coastal grassland. In contrast, more than half of the soil organic C

Table 2

Size class characteristics of compost applied at both grassland experimental sites. Values are means with standard errors of replicate samples in parentheses.

Compost size class	Fraction of total	% C	% N	C:N
<1 mm	43 (1)	20.9 (0.05)	1.85 (0.01)	11.3 (0.1)
1–2 mm	35 (1)	23.7 (0.49)	1.84 (0.03)	12.9 (0.2)
2–4.75 mm	16 (3)	22.6 (0.81)	1.53 (0.11)	14.3 (0.4)
>4.75 mm	6.0 (0.5)	28.5 (1.7)	1.52 (0.01)	18.7 (1.2)
Rocks	0.8 (0.4)			
Synthetics	1.3 (0.6)			
Bulk	100	22.7 (0.5)	1.78 (0.05)	12.7 (0.2)

was stored in the light fractions at the valley grassland ($p < 0.001$). Carbon and N content of the OLF and HF were positively correlated to clay content in the coastal grassland ($r^2 = 0.40$ and 0.37 for OLF C and N content; $r^2 = 0.57$ and 0.58 for HF C and N content; $p < 0.01$ in all cases). All three fractions were negatively correlated with sand content at this site ($r^2 = 0.30$, 0.54 , and 0.60 for FLF, OLF, and HF C content; $r^2 = 0.30$, 0.52 , and 0.64 for FLF, OLF, and HF N content; $p < 0.05$ in all cases).

3.4. DRIFT spectra of soil density fractions

There were qualitative similarities in peak intensities between the FLF, OLF, and compost with less intense spectra in the aliphatic band A region relative to the band B region. Compared to these fractions, the DRIFT spectra of the HF showed a noticeable diminution of absorbance intensities of the aliphatic methylene groups (Fig. 3). For the control soil fractions, the B:A* ratio of the FLF and OLF was significantly greater for valley grasslands compared to coastal grasslands ($p < 0.05$). In contrast, the coastal grassland HF showed significantly greater B:A* ratio compared to the valley

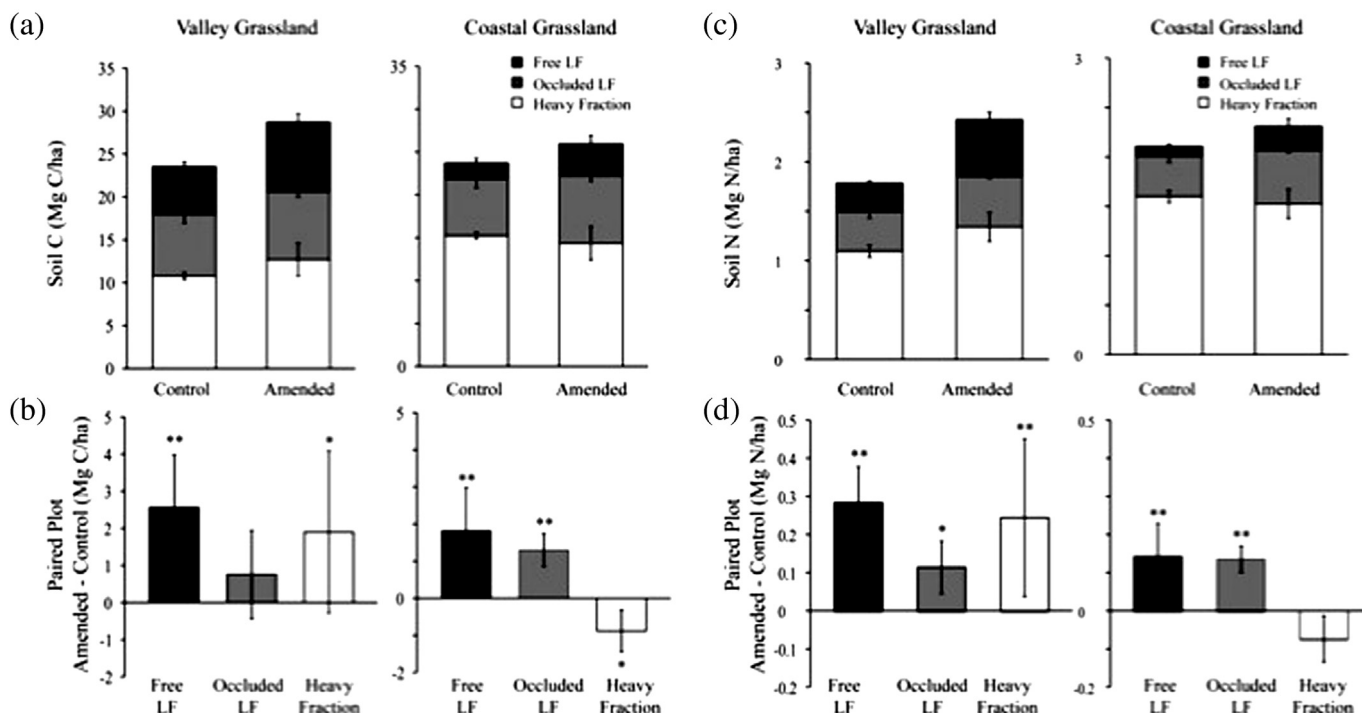


Fig. 2. (a) The contribution of three soil fractions to total soil organic C content from 0 to 10 cm depth for control and amended soils in two grassland ecosystems (mean \pm 1 standard error, $n = 3$). (b) The treatment effect on C storage for each soil fraction shown as differences between each pair of amended and control plots in year 3 ($n = 3$). (c) The contribution of three soil fractions to total soil N content from 0 to 10 cm depth. (d) The treatment effect on soil N content for each soil fraction shown as paired plot treatment differences. * Indicates $p < 0.1$; ** Indicates $p < 0.05$ for differences between paired plots.

Table 3

Mass and C and N composition of soil density fractions from control and organic amended soils from two rangeland ecosystems.

Fraction	Grassland	Treatment	Percent of bulk soil mass	C (%)	C (Mg C/ha)	N (%)	N (Mg C/ha)	C:N
Free light fraction	Valley	Control	3.2 (0.1)**	21.8 (1.0)	5.57 (0.54)**	1.14 (0.01)**	0.30 (0.02)**	19.2 (0.7)**
		Amended	4.0 (0.2)**	22.2 (0.4)	8.13 (1.00)**	1.56 (0.04)**	0.57 (0.07)**	14.2 (0.2)**
	Coastal	Control	0.9 (0.2)**	21.3 (1.1)	1.88 (0.10)**	1.13 (0.03)**	0.10 (0.01)**	18.8 (1.2)**
		Amended	1.8 (0.7)**	22.9 (1.4)	3.70 (1.22)**	1.44 (0.03)**	0.24 (0.10)**	15.8 (0.9)**
Occluded light fraction	Valley	Control	3.3 (0.4)	28.5 (3.3)	7.15 (1.00)	1.58 (0.22)**	0.40 (0.06)*	18.0 (0.8)**
		Amended	2.9 (0.4)	31.1 (1.3)	7.91 (0.62)	2.00 (0.17)**	0.51 (0.02)*	15.4 (0.6)**
	Coastal	Control	2.2 (0.7)**	31.7 (1.6)	6.58 (1.64)**	1.90 (0.09)**	0.41 (0.11)**	16.5 (0.5)**
		Amended	2.5 (0.6)**	33.0 (1.1)	7.87 (2.00)**	2.25 (0.09)**	0.54 (0.14)**	14.6 (0.1)**
Heavy fraction	Valley	Control	93.5 (0.4)	1.45 (0.10)	10.8 (0.4)*	0.15 (0.01)**	1.10 (0.06)**	9.9 (0.4)*
		Amended	93.1 (0.2)	1.48 (0.11)	12.7 (1.9)*	0.16 (0.01)**	1.34 (0.15)**	9.4 (0.4)*
	Coastal	Control	96.9 (0.8)**	1.64 (0.40)	15.2 (2.0)*	0.17 (0.04)	1.60 (0.18)	9.5 (0.2)
		Amended	95.7 (1.1)**	1.58 (0.24)	14.4 (2.4)*	0.17 (0.02)	1.53 (0.24)	9.4 (0.2)

* Indicates significant within-site treatment differences of each fraction at $p < 0.10$.** Indicates significant within-site treatment differences of each fraction at $p < 0.05$.

grassland HF ($p < 0.05$; Fig. 4). At both sites, the B:A* ratio of HF was significantly greater than that of either the FLF and OLF ($p < 0.0001$ at both sites), The B:A* ratio of the compost amendment was significantly less than the HF and greater than the FLF and OLF.

The amended soil fractions generally had a diminished intensity of band A groups and more intense peaks of band B groups relative to paired control soils. The mean B:A* ratios of the FLF in amended soils at the valley grassland and the FLF and OLF in amended soils at the coastal grassland were greater than those from control soils (Fig. 4). The B:A* ratio of these fractions showed similarities to the compost amendment, suggesting incorporation of the amendment into both the FLF and physically-protected OLF in the 0–10 cm soil depth.

4. Discussion

4.1. Changes to soil C and N with organic matter amendments

Organic matter amendments add C and N to the soil surface; this research showed that amendment C and N are also physically protected by incorporated into soil aggregates over relatively short time periods. We measured an increase of 18% in C content and 30% in N content in the valley site (0–10 cm) and an increase of 9% in both C and N contents (0–10 cm) at the coastal site over three years following a single application of compost. It is important to note that compost litter was carefully removed prior to analysis, so that our results represent a true increase in mineral soil C and N. Organic

matter amendments have been shown to increase bulk soil C pools (generally including associated amendment litter) in the few existing short-term amendment experiments conducted on pastures and grasslands (e.g. Lynch et al., 2005; Cabrera et al., 2009). In a four-year study, soil C increased by up to 9.7 Mg C/ha in a perennial grassland after receiving compost amendments for two consecutive years in combination with seeding of legumes (Lynch et al., 2005). Similarly, manure amendments to grassland in New Mexico increased SOM by 34% after three years (Cabrera et al., 2009). The application of composted biosolids to a semi-arid grassland in Colorado resulted in significant increases in soil C, as well as increases in plant productivity, microbial biomass, and net N mineralization rates (Pascual et al., 1999; Sullivan et al., 2006; Fernandez et al., 2007; Ippolito et al., 2010; Kowalijow et al., 2010), the effects of which were evident for at least 14 years following a single application (Ippolito et al., 2010). Previous work at our sites showed that the amendment stimulated C losses through elevated soil respiration, but that losses were offset by larger C gains through increased above- and belowground net primary productivity (Ryals and Silver, 2013).

4.2. Soil density fractions

Changes in the concentrations and mass of C and N in density fractions can provide an early indication of overall fate of the amendment and the long-term C storage potential (He et al., 2008). Using a physical fractionation procedure, we detected greater soil C

Table 4DRIFT peak heights of aliphatic methylene groups present at wavenumbers 2920 and 2852 cm^{-1} (Band A), carbonyl and carboxyl groups present at wavenumbers 1653 and 1400 cm^{-1} (Band B), and the ratio of Band B: Band A absorbance in soil density fractions and in bulk compost.

Grassland	Treatment	Absorbance at				Ratio of Band B: Band A %C ⁻¹
		Wavenumber 2920 $\text{cm}^{-1,a}$	Wavenumber 2853 $\text{cm}^{-1,a}$	Wavenumber 1653 $\text{cm}^{-1,b}$	Wavenumber 1400 $\text{cm}^{-1,b}$	
Valley	Control	0.571 (0.208)	0.054 (0.038)	1.384 (0.166)	0.528 (0.060)	0.16 (0.06)
	Amended	0.607 (0.241)	0.102 (0.054)	1.607 (0.204)	0.569 (0.079)	0.22 (0.09)
Coastal	Control	1.146 (0.661)	0.211 (0.161)	1.613 (0.702)	0.502 (0.174)	0.11 (0.05)
	Amended	0.797 (0.334)	0.065 (0.032)	1.231 (0.166)	0.463 (0.085)	0.14 (0.06)
Valley	Control	0.530 (0.343)	0.364 (0.285)	1.569 (0.227)	0.723 (0.131)	0.13 (0.05)
	Amended	0.579 (0.245)	0.273 (0.142)	1.798 (0.202)	0.828 (0.103)	0.12 (0.03)
Coastal	Control	0.623 (0.368)	0.508 (0.313)	1.559 (0.248)	0.622 (0.073)	0.07 (0.02)
	Amended	0.577 (0.433)	0.355 (0.206)	1.505 (0.180)	0.673 (0.100)	0.10 (0.03)
Valley	Control	0.040 (0.019)	0.032 (0.013)	0.065 (0.011)	0.006 (0.002)	1.04 (0.42)
	Amended	0.043 (0.017)	0.028 (0.012)	0.085 (0.028)	0.009 (0.004)	1.29 (0.50)
Coastal	Control	0.031 (0.011)	0.030 (0.013)	0.096 (0.020)	0.015 (0.004)	1.83 (0.69)
	Amended	0.038 (0.018)	0.021 (0.006)	0.099 (0.014)	0.014 (0.004)	1.93 (0.73)
–	–	0.240 (0.062)	0.178 (0.061)	1.744 (0.122)	0.594 (0.071)	0.33 (0.11)

^a Indicates groups that are included in Band A.^b Indicates groups that are included in Band B.

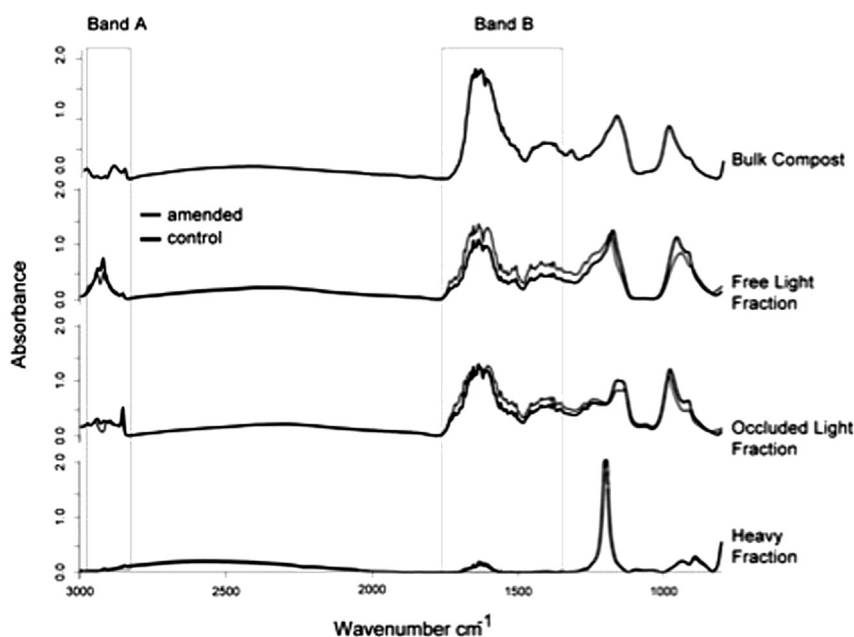


Fig. 3. Representative DRIFT spectra of control and amended soil density fractions and of bulk compost. Spectra are means of field replicates ($n = 3$). Assignments for evaluated bands are shown in Table 2.

and N storage in the amended plots, particularly in the FLF and OLF. After three years, compost amendment increased soil C stored in the light fractions by 3.31 ± 1.64 Mg C/ha and 3.11 ± 1.08 Mg C/ha, or by 26% and 37%, at the valley and coastal grassland, respectively. The soil C sequestration rates estimated from the density fractionation procedure were greater than the average treatment increase of 1.92 ± 0.65 Mg C/ha based on the three-year mass balance of annual C gains (*i.e.* above- and belowground net primary production) and losses (*i.e.* heterotrophic soil respiration) at these sites (Ryals and Silver, 2013). This suggests that the processes of amendment C stabilization and indirect increases in C through enhanced plant production are additive.

Soil C and N contents were positively correlated with clay content at the coastal site. This may have resulted from greater aggregation with increasing clay content (Bronick and Lal, 2005) or a general increase in fertility and soil moisture associated with more finely textured soils (Dalal and Mayer, 1986). Stabilization of C in amended agricultural soils has been attributed to the promotion of physical protection of organic C in soil aggregates (Six et al., 2000; Chivenge et al., 2007; Majumder et al., 2010; Majumder and Kuzyakov, 2010) and greater physico-chemical association of organic C with clay- and silt-sized mineral particles (Razafimbelo et al., 2008). Amendments can improve soil aggregation (Pagliai et al., 1981; Aggelides and Londra, 2000; Pagliai et al., 2004), which may have contributed to the greater soil C storage in the OLF of amended plots. When occluded in soil aggregates, soil organic C is generally less accessible to decomposer organisms and thus more protected from decomposition (Dungait et al., 2012). In similar grassland types, the turnover of these intermediate fractions determined by radiocarbon measurements was on the decadal-scale, ranging with soil age from 18 to 42 years (Baisden et al., 2002). Carbon inputs from organic matter amendments can also enhance soil microbial activity (García-Gil et al., 2000; Poulsen et al., 2013) and produce microbially-derived compounds that are the primary constituents of stable SOM (Grandy and Neff, 2008). This can further contribute to the formation of long-term SOM storage (Bradford et al., 2013).

The additional C found in the FLF and OLF likely originated from two possible sources. Some of the additional soil C in amended plots was due to incorporation of the C from the amendment material. A second source for the additional C found in amended soil may have been above- and belowground plant litter. Many grasslands are limited by N and water availability (Dukes et al., 2005; Harpole et al., 2007; Chou et al., 2008). Studies of inorganic N fertilization to grasslands have consistently shown a positive response of plant production to fertilization, which is often accompanied by increased soil C storage (Derner and Schuman, 2007). Compost amendments in this study added 129 g total N/m². Organically bound N in compost is likely to be less immediately available for plant uptake compared to inorganic N fertilizers or many fresh plant litters. However, net primary productivity generally increases with compost additions (Lynch et al., 2004;

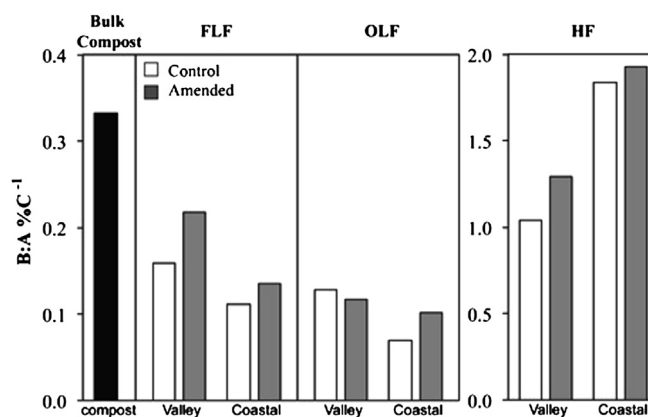


Fig. 4. Ratio of Band B absorbance to Band A absorbance from FT-IR spectra, normalized to carbon concentration, of free-light (FLF), occluded-light (OLF), and heavy fractions (HF) from valley grassland and coastal grassland experimental plots. White bars represent mean values from control soils. Gray bars represent mean values from amended soils. The black bar represents the mean value of bulk compost. Standard errors are provided in Table 4.

Cabrera et al., 2009; McFarland et al., 2010). Ryals and Silver (2013) measured cumulative increases of 2.0 ± 0.8 Mg C/ha and 4.7 ± 0.7 Mg C/ha in net primary production (sum of above- and belowground production) at the coastal and valley grassland, respectively, over three years following the single compost amendment. The positive plant production response showed no signs of diminishing over time. The large added N pool likely acts as a slow release fertilizer, which may further enhance C inputs to the soil.

Contrary to our expectations, the increase in N concentration was greater relative to the increase in C concentration in soil fractions of the amended plots, leading to a decrease in the C:N ratio of the light fractions with amendment. After three years, compost amendments increased soil N stored in the light fractions by 0.38 ± 0.10 Mg N/ha and 0.27 ± 0.10 Mg N/ha, or by 54% and 53%, at the valley and coastal grassland, respectively. We hypothesized that N would be lost faster than C following compost amendment (i.e. lead to higher C:N ratios) due to more rapid N mineralization during decomposition (Parton and Silver, 2007). Narrower C:N ratios are also a possible result of microbial processing of C during decomposition (Rodionov et al., 2000; Six et al., 2001). Nitrogen may be more strongly complexed in composted organic matter relative to fresh plant litter leading to the patterns observed (Lynch et al., 2006).

Compost is generally assumed to be more recalcitrant than fresh litters, and thus chemically resistant to decomposition. The notion of inherent recalcitrance of organic material is currently being debated (Kögel-Knabner et al., 2008; Marschner et al., 2008; Schmidt et al., 2011; Dungait et al., 2012) but functional group chemistry plays a role in solubility, organo-mineral interactions, and other processes related to organic matter reactivity. By combining chemical characterization with physical fractionation, we can begin to assess the effect of compost amendments on the degree of physical and chemical protection of organic matter in soils. Qualitative and semi-quantitative FTIR analyses have been used to infer potential differences in turnover dynamics of, for example, aggregate-associated and/or soluble organic matter fractions separated from different soil types or from different management treatments (Gerzabek et al., 1997; Kaiser et al., 2007; Simonetti et al., 2011; Kaiser et al., 2012). In this study, DRIFT spectroscopic analyses of organic matter fractions revealed differences in the functional group composition between the two light fractions and the heavy fraction in control soils at both grassland sites. Compared to OLF, the FLF consisted of greater absorbance intensities of aliphatic methyl and methylene groups, lower absorbance intensities of carboxyl and carbonyl groups, yet similar B:A* ratios. The HF consisted of diminished intensities of aliphatic methylene groups compared to the light fractions (Ouatmane et al., 2000). These patterns are consistent with the hypothesis that the fractions represent varying degrees of decomposition with the $FLF < OLF < HF$ (Poirier et al., 2005). Organic matter amendments altered the functional group composition of the FLF and OLF. The B:A* ratios of the both light fractions were greater in amended versus control soils. At both grasslands, the ratio of B:A* of the FLF and OLF started to reflect the respective ratio of the compost, suggesting incorporation of the amendment into these fractions by year three of the study.

4.3. Implications for long-term C and N storage

Studies analyzing the effect of organic matter amendments on C and N dynamics in grassland soils are scarce compared to those in croplands. Our results show that a single application of compost to two grasslands resulted in soil C sequestration rates of over 3 Mg C/ha over three years with concomitant increases in soil N. At the

Rothamsted Broadbalk Experiment, the longest running agricultural experiment, farmyard manure was added to winter-wheat cropping fields annually for over 150 years (Blair et al., 2006). Powlson et al. (2012) found that the most rapid rates of soil C gain occurred during the first 20 years of application (1.0 Mg C ha^{-1} yr^{-1}), and then slower rates thereafter (0.2 Mg C ha^{-1} yr^{-1} during the last 20 years).

It has been hypothesized that soils have a maximum C storage potential (i.e. C-saturation capacity) determined by physicochemical properties of the soil (Six et al., 2002). The maximum C storage potential has not been determined for the valley or coastal grasslands studied here, but at least two lines of evidence suggest that these soils are considerably below their C-saturation level, if some exists. First, the shift from perennial-dominated grasslands to annual grasslands in California in the 1800s may have caused a decline in soil C stocks of approximately 40 Mg C/ha in the top 50 cm of soil (Koteen et al., 2011); C stocks may still be declining (Chou et al., 2008; Ryals et al., 2013). Thus, there is theoretical potential for these systems to at least regain the soil C that has been lost to the atmosphere through management activities. Changes in plant community composition, and particularly an increase in perennial plant cover, have been proposed as a means to increase soil C storage (Fornara and Tilman, 2008). Over the course of this study, we did not observe an increase in perennial vegetation (Ryals et al. 2013), thus observed changes can be attributed directly to C inputs from the amendment and indirectly from enhanced plant production. Second, soil organic C stocks vary widely across grasslands in California, ranging from 28 to 137 Mg C/ha in the top 50 cm of soil (Silver et al., 2010). The background soil C content of the valley and coastal grasslands fall well below the regional average at approximately 53 and 57 Mg C/ha to 50 cm depth, once again supporting the hypothesis that these soils have considerable capacity for additional C storage. The detected incorporation of new C into the SOM fractions in combination with the previously determined sustained boost in plant production and soil litter input emphasize the potential of compost amendments to grassland soils for C sequestration. Further, these results suggest that increases in C oxidation associated with compost amendment is likely derived primarily from enhanced plant litter inputs, rather than rapid decomposition of the compost (Ryals and Silver, 2013).

5. Conclusions

Three years after a single application of composted green waste, we found that soil C and N increased by 26% and 54%, respectively, at the valley grassland and by 37% and 53%, respectively, at the coastal grassland. The additional C and N was stored in the FLF and physically-protected OLF, and was likely derived from a combination of direct inputs of compost C and increases in plant production (Ryals and Silver, 2013). The C:N ratio and the proportion of intensities of oxygen-containing carbonyl and carboxyl functional groups to aliphatic methyl and methylene functional groups increased in the light fractions of the amended soils, suggesting incorporation and retention of amendment. Our results indicate that degraded grassland soils may be highly responsive to C sequestration through amendment management strategies. The inclusion of amendment C and N to physically-protected pools suggests that short-term or single management events may have a long-lasting impact on ecosystem C and N dynamics.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2013.09.011>.

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