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Research article

Initial aggregate formation and soil carbon storage from lipid-extracted algae amendment

Katie Lewis[1,*], Jamie Foster[2], Frank Hons[3] and Thomas Boutton[4]

[1] Texas A&M AgriLife Research, Texas A&M University, Lubbock, TX, USA
[2] Texas A&M AgriLife Research, Texas A&M University, Beeville, TX, USA
[3] Soil and Crop Sciences Department, Texas A&M University, College Station, TX, USA
[4] Ecosystem Science Management Department, Texas A&M University, College Station, TX, USA

*** Correspondence**: Email: katie.lewis@ag.tamu.edu; Tel: +806-746-6101; Fax: +806-746-6528.

Abstract: Soil organic C (SOC) storage results when organic matter inputs to soil exceed losses through decomposition, and is strongly influenced by organic matter effects on soil aggregation. We evaluated the initial effects of lipid-extracted algae (LEA), a byproduct of biofuel production, on soil aggregate formation and SOC storage. In situ field incubations were conducted by amending soil with (1) 1.5% LEA, (2) 3.0% LEA, (3) 1.5% LEA + 1.5% wheat straw (WS) and (4) soil plus inorganic N (140 kg ha^{-1} NH_4NO_3) and P [112 kg ha^{-1} $Ca(H_2PO_4)_2 \cdot H_2O$] as the control. Soil samples were collected 0, 3, 6, 9, and 12 months after treatment application at 0–5, 5–15, and 15–30 cm. Soil was separated into macroaggregate (>250 µm), microaggregate (250–53 µm), and silt and clay (<53 µm) fractions by dry-sieving, and mean weight diameter was calculated. Soils and soil fractions were analyzed to determine C concentrations and associated $\delta^{13}C$ values. Mean weight diameter 12 months after 3.0% LEA application was greater than the 1.5% LEA + 1.5% WS addition at the 5–15 cm depth. Soil amended with 1.5% LEA, 3.0% LEA or 1.5% LEA + 1.5% WS resulted in greater SOC after 12 months for all soil size fractions and depths. $\delta^{13}C$ indicated that most LEA-C was initially associated with the silt and clay fraction, but later became more strongly associated with the macro- and microaggregate fractions after 12 months. Soil application of LEA enhanced initial aggregate formation and SOC storage by increasing aggregate MWD and macro- and microaggregate associated SOC over time. As the world population grows and resources become more limited, use of alternative energy sources, soil conservation, and environmental protection must be top research priorities. Our research emphasized all three and demonstrated that LEA can enhance soil structure and C storage.

Keywords: soil organic carbon; carbon storage; aggregate stability; soil amendment; algal residue; bioenergy feedstock; wheat straw residue; organic matter; soil structure; stable carbon isotopic composition

1. Introduction

The largest C pool within the terrestrial C cycle is soil organic C (SOC) and its storage is the net outcome of organic matter (OM) inputs to soil and losses through decomposition [1,2]. Soil organic C strongly influences other soil properties and processes, such as water holding and cation exchange capacities, retention of nutrients in the root zone, buffering against pH change, ability to chelate and form complex ions, and the maintenance of soil physical structure via aggregate formation [3]. In agroecosystems, the maintenance of these soil characteristics reduces the potential for soil degradation, erosion and compaction, increases nutrient availability to plants and microorganisms, and favors the capacity for C storage [4].

Long-term SOC stabilization and short-term nutrient cycling are also influenced by dynamics of aggregate formation and breakdown over time [5,6]. Organic amendments can enhance soil aggregate formation by providing active organic materials, such as particulate OM, which act as nucleation sites and binding agents to facilitate aggregate formation [7,8]. Six et al. [9] suggested that adding crop residues promotes OM stabilization through the binding of primary soil particles and old microaggregates into new macroaggregates. Depending on the quality or biochemical characteristics, such as C:N ratio and lignin content of organic materials, it may be possible to maintain or improve soil physical and chemical properties as a result of increased SOM, and consequently, enhanced microbial activity and aggregate formation.

Jastrow et al. [10] observed an increase of macroaggregates resistant to slaking under long-term pasture grasses compared to corn (*Zea mays* L.). A monoculture study by Wright and Hons [11] reported aggregation to be generally greater for wheat (*Triticum aestivum* L.) than sorghum [*Sorghum bicolor* (L.) Moench] and soybean [*Glycine max* (L.) Merr.]. Crop residues having low N contents and greater C:N ratios, such as wheat straw (WS), will usually decompose at slower rates than residues with greater N and narrower C:N ratios [12,13]. Slower decomposition often leads to increased SOM, aggregate formation, and SOC stability.

In a long-term incubation study (392 d), greater organic C accumulation and stabilization and N availability were determined when soil was amended with lipid-extracted algae (LEA) compared to soil amended with WS [14]. Algal residue or LEA following lipid extraction of algae cultivated as a bioenergy feedstock is one of the many possible coproducts that might be used as a soil amendment for agricultural production. Compared to lignin in wheat straw, LEA originating from the algal species *Nannochloropsis salina* contained a larger stable C fraction, which likely originated from aliphatic macromolecules known as algaenans which are more resistant to decay than other macromolecular compounds derived from proteins, polysaccharides, and even lignin [15,16]. The high concentration of basic cations (Ca^{2+}, Mg^{2+}, and Na^{+}) in LEA also contributed to increased soil salinity, with electrical conductivity (EC) being nearly five times greater in LEA-treated soil compared to untreated soil 14 d following LEA application. High concentrations of Na^{+} in LEA may potentially impair soil structure, and reduce aggregate formation and subsequent C storage.

Aggregates can contain OM of various origins, composition and degree of microbial degradation, and thus, add to the difficulty of studying the role of organic amendments in aggregate formation and the ensuing effect of aggregate turnover on soil C stabilization. However, natural differences in the stable C isotopic composition ($^{13}C/^{12}C$) of agricultural soils and organic amendments can provide a useful approach for tracing and identifying the sources of C sequestered in aggregates and SOM fractions [17,18]. Carbon in nature is comprised of 98.89% ^{12}C and 1.11% ^{13}C [19]. As a result of C isotope fractionation during physical, chemical, and metabolic processes, considerable C isotope variation exists between different components of the environment [20,21], and this variation can be utilized to trace pathways of C flow through the environment. For example, the $^{13}C/^{12}C$ ratio (or $\delta^{13}C$ value) of SOC relates closely to the ^{13}C content of the plant or microbial material it originated from [22].

Cool- (C_3) and warm-season (C_4) plant species discriminate against $^{13}CO_2$ during photosynthesis to different degrees. C_3 species discriminate against $^{13}CO_2$ to a greater extent [23], making it possible to determine the relative contribution of C_3 and C_4 plant vegetation to SOM [24]. Growing C_4 plants on soil that has previously been under C_3 vegetation, or vice versa, can be used as an in situ labeling of the incorporated SOM [25]. Carbon isotope tracers allow for the quantification of the rate of C losses from the original vegetation and the simultaneous accumulation of new C from the current vegetation or recent organic material addition.

Physical size-fractionation of soil aggregates in conjunction with isotopic analyses ($\delta^{13}C$) of those fractions have been used to: (1) determine where OC derived from isotopically distinct sources is stored relative to aggregate structure; (2) identify sources of SOC; (3) quantify turnover rates of SOC in specific soil fractions, and, (4) evaluate OM quality [26]. Jastrow et al. [10] showed that recent OM inputs are found mostly in larger soil aggregates, making it more susceptible to decomposition because macroaggregates are more likely to be destroyed by agricultural practices compared to microaggregates [7], but in perennial pasture systems this may not be the case.

The objective of our research was to determine the initial effects of LEA, a byproduct of biofuel production and a potential organic amendment, on soil aggregate formation and SOC storage by: (1) determining the mean weight diameter of soil aggregates with time after LEA incorporation; (2) determining SOC storage within aggregates; and, (3) delineating the C sources and distribution in aggregate fractions by utilizing the natural abundance of ^{13}C of Parrita soil and LEA and WS materials.

2. Materials and Methods

2.1. Site Description and Experimental Design

The study was conducted at the Texas A&M Agrilife Research Station near Beeville, TX (28º27'30", 97º42'21.78", 75.9 m). According to the National Centers for Environmental Information, the mean annual temperature and mean annual precipitation (1981 to 2010) for this semi-arid environment are 21 ºC and 81 cm, respectively. Soil at this location is characterized as a Parrita series (loamy, mixed, superactive, hyperthermic, shallow Petrocalcic Paleustoll), and is a sandy clay loam with a pH of 6.9.

The study was designed as a split-plot and arranged in a randomized complete block design with sampling time as the main plot and soil amendment as the split plot. In situ field incubations

were conducted in polyvinyl chloride (PVC) columns measuring 10 cm (i.d.) × 33 cm. The bottoms of columns were capped, but drainage holes were drilled in caps to allow water to drain from soil columns. Weights of empty columns and soil-filled columns were measured.

Soil columns were removed at different times (0, 3, 6, 9, and 12 months) after treatment application and destructively sampled. Samples collected 24 hours after treatment application are referred to as 0 months. Treatments included: (1) 1.5% LEA, (2) 3.0% LEA, (3) 1.5% LEA + 1.5% WS, and (4) a control of soil plus inorganic N (140 kg ha^{-1} NH_4NO_3) and P [112 kg ha^{-1} $Ca(H_2PO_4)_2 \cdot H_2O$]. The combination of four replications per sampling time (5) x soil amendment treatment (4), totaled 80 columns. Treatments were prepared by mixing the designated rate of inorganic fertilizer, LEA or WS with sieved (<2 mm) Parrita soil on a dry weight basis (g g^{-1}). The lowest 10 cm of each column was filled with unamended soil and the top 20 cm with amended soil and gently packed so that soil bulk density was approximately 0.8 g cm^{-3}. Columns were then placed and securely packed within excavated holes measuring 11 cm wide and 30 cm deep at a field site near the soil collection location at the Beeville Research Station.

2.2. Soil, lipid-extracted Algae, and Wheat Straw Characterization

Once columns were removed, soil was sampled from three depth increments (0–5, 5–15, and 15–30 cm) for measuring soil aggregate-size fractions, isotopic and elemental concentrations of SOC, pH and electrical conductivity (EC) and sampled from 0–15 and 15–30 cm for SOC and total N. Soil, LEA, and WS were analyzed for OC and total N by a combustion procedure [27-29]. Lipid-extracted algae and WS mineral concentrations (Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn) were determined by ICP analysis of nitric acid digests [30,31]. Concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) of LEA and WS were measured sequentially using an ANKOM 200 Fiber Analyzer (Ankom Technologies, Macedon, NY) based on the method of Van Soest et al. [32] and AOAC [33] (method 973.18) and lignin was sequentially measured after ADF using the Ankom [14,34] method. Initial extractable P, K, Ca, Mg, S, and Na of Parrita soil were measured using the Mehlich III procedure [35,36] with analysis by ICP; micronutrients (Cu, Fe, Mn, and Zn) were assessed by extraction with DTPA-TEA, followed by ICP analysis [37].

After removing columns from the field, wet and dry weights were determined prior to and after oven drying (65 ºC) to constant weight. Soil samples for aggregate-size fractionation were gently crushed and sieved (<4 mm) prior to separating random 50-g aliquots into three size fractions [>250 μm (macroaggregates), 250–53 μm (microaggregates), and <53 μm (silt and clay)] using a rotary sieve-based dry sieving method [38,39]. Mean weight diameter (MWD) was calculated as a weighted average of the soil size fraction percentages. Separated size fractions were weighed and ground using a ring and puck mill (<150 μm) prior to elemental and isotopic analyses.

Sub-samples of soil collected at 0, 3, 6, and 12 months were ground with a flail grinder (<2 mm). The pH and EC of soil, LEA, and WS were determined in a 1:2 soil or residue to water extract using deionized water with EC determinations made using a conductivity probe [40]. Sub-samples for soil total C and N were further ground (<150 μm) using a ring and puck mill prior to weighing and combustion analysis described previously.

Soil aliquots of 24 or 30 mg, depending on C concentration, of the three size fractions from the 0–5, 5–15, and 15–30 cm depths were weighed for elemental and isotopic analysis of SOC and $\delta^{13}C$, which were performed in the Stable Isotopes for Biosphere Science (SIBS) Laboratory, Texas A&M

University, College Station, TX. LEA and WS were also measured for elemental C and $\delta^{13}C$. A Costech Elemental Combustion System interfaced with a Thermo Scientific Delta V Advantage mass spectrometer operating in continuous flow mode was used to determine isotope ratios relative to the Vienna Pee Dee Belemnite (V-PDB) standard [41]. Carbon isotope ratios were expressed as per mil (‰) using the standard delta notation (δ):

$$\delta = (R_{SAMPLE} - R_{STD})/R_{STD}) \times 10^3, \quad (1)$$

where R_{SAMPLE} is the $^{13}C/^{12}C$ of the sample and R_{STD} is the $^{13}C/^{12}C$ ratio of the V-PDB standard. Quality control was performed using calibration curves, which were derived using USGS standards of glutamic acid-40 ($\delta^{13}C$ = −26.39‰, $\delta^{15}N$ = −4.52‰) and USGS glutamic acid-41 ($\delta^{13}C$ = 37.63‰). Plant material of corn ($\delta^{13}C$ = −12.78‰) and a lab plant standard ($\delta^{13}C$ = −39.88‰) were analyzed as internal standards to determine the accuracy and precision of isotopic analysis.

The relative proportions of SOC derived from the LEA (F_C) vs. the native soil C were estimated by mass balance:

$$F_C = (\delta_{SAMPLE} - \delta_{SOIL})/(\delta_{AMENDMENT} - \delta_{SOIL}), \quad (2)$$

where δ_{SOIL} was the $\delta^{13}C$ value of native soil C at the start of the experiment, δ_{SAMPLE} was the $\delta^{13}C$ value within a soil size fraction at given sampling time, and $\delta_{AMENDMENT}$ was the original $\delta^{13}C$ value of LEA or the weighted average of LEA and WS used for the 1.5% LEA + 1.5% WS treatment. The percentage of SOC derived from LEA and LEA plus WS (*amendment-C*, %) within soil size fractions was calculated as:

$$amendment{-}C, \% = F_C \times 100, \quad (3)$$

2.4. Statistical Analysis

The effects of soil amendment on measured parameters were analyzed using a linear mixed analysis of variance (ANOVA) procedure at a significance level of $P < 0.05$ using SAS version 9.3 [42]. Treatment was considered a fixed effect while replication was a random effect. Means of significant effects were separated using Fisher's protected LSD at $P < 0.05$.

3. Results

3.1. Soil, Lipid-extracted Algae, and Wheat Straw Characterization

The soil collected from the Texas A&M Agrilife Research Station in Beeville, TX, and used for this in situ field experiment is classified as Parrita sandy clay loam with a pH of 6.9 and EC of 0.2 dS m^{-1} (Table 1). Soil OC and TN of Parrita soil were 9.6 g kg^{-1} and 1.4 g kg^{-1}, respectively. This soil was high in extractable P (62 mg kg^{-1}), Ca (2667 mg kg^{-1}), and Mg (278 mg kg^{-1}), very high in K (314 mg kg^{-1}), moderate in S (12 mg kg^{-1}), and very low in Na (59 mg kg^{-1}) based on ratings of the Texas A&M AgriLife Extension Soil, Water and Forage Testing Laboratory. Extractable Fe (6 mg kg^{-1}), Cu (0.5 mg kg^{-1}), and Mn (16 mg kg^{-1}) were rated as high, while Zn (0.5 mg kg^{-1}) was moderate to high.

Table 1. Soil, LEA, and WS chemical characteristics [14].

			Organic	Total	Soil extractable nutrients[†] & LEA/WS total minerals[‡]									
	pH	EC	C	N	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	----	dS m^{-1}	g kg^{-1}		mg kg^{-1}									
Soil														
Parrita	6.9	0.2	9.6	1.4	62	314	2667	278	12	59	6	0.5	16	0.5
Amendments														
LEA	9.9	33	343	32	4339	6997	62666	7212	9282	52922	3664	29	79	14
WS	6.6	3.4	404	8	800	14400	3200	800	992	533	39	20	17	5

[†]Soil nutrients are Mehlich III (P, K, Ca, Mg, Na, and S) and DTPA (Fe, Zn, Mn, and Cu) extractable.

[‡]Lipid extracted algae (LEA) and wheat straw (WS) were analyzed for total minerals.

The pH and EC of LEA were 9.9 and 32.5 dS m^{-1}, respectively and 6.6 and 3.4 dS m^{-1} for WS [14] (Table 1). Total N was four times greater in LEA (32 g kg^{-1}) than in WS (8 kg kg^{-1}), but WS-C (404 g kg^{-1}) was approximately 1.5 times greater than that of LEA (343 g kg^{-1}) [14]. The C:N ratio of LEA and WS were 10.8 and 50.5, respectively. Lipid-extracted algae contained greater mineral concentrations than WS, except for K. Both WS and especially LEA should potentially contain sufficient quantities of nutrients to support most agronomic crops when applied at sufficiently high rates.

Fiber analysis indicated potential differences in degradability/stability between LEA and WS. As reported by Rothlisberger-Lewis et al. [14], values of NDF, ADF, and lignin or the most stable C fraction in LEA were 29.1%, 17.3%, and 13.4% (DM basis), respectively, while these components represented 71.9%, 43.4%, and 3.5% (DM basis) of WS, respectively.

3.2. Amendment Effects on Bulk Soil

3.2.1. Soil pH and electrical conductivity

At 0 months regardless of depth, LEA-amendments resulted in similar soil pH values that were all greater than the control (Table 2), likely due to significant quantities of basic cations added by LEA (Table 1). By 12 months, pH had a tendency to decrease for all LEA-treatments and the control, possibly due to acidification from subsequent mineralization reactions. No differences in pH were observed between treatments at 12 months at 0–5 cm depth (P = 0.25). However, soil pH at 12 months was similar for all LEA-amendments within the 5–15 and 15–30 cm depths, with LEA-amended soil still having pH values greater than the control.

Soil EC was greatest with 3.0% LEA at 0 and 12 months for all depths, while at 0 months, all LEA treatments also resulted in significantly greater EC than the control (Table 3). As depth and time increased, the magnitude of the difference in EC between all treatments decreased. The 3.0% LEA treatment had the greatest measured EC of 4.3 dS m^{-1} in the 0–5 cm depth at 12 months. Regardless of depth at 12 months, soil EC was similar for the control and all treatments except 3.0% LEA.

3.2.2. Soil C Dynamics

Organic C of bulk soil 0 months after treatment application within the 0–15 cm depth was greatest with 1.5% LEA + 1.5% WS (16.6 g kg^{-1}), followed by 3.0% LEA (13.7 g kg^{-1}), and then 1.5% LEA (11.2 g kg^{-1}) and the control (+N,+P) (9.6 g kg^{-1}) (Figure 1a). Soil OC with 1.5% LEA + 1.5% WS was greatest likely because of the greater C concentration of WS (Table 1). Similar trends generally were exhibited throughout the remainder of the study; however, significant differences in SOC between 1.5% LEA + 1.5% WS and 3.0% LEA at 3, 6, and 12 months were not observed, but these treatments still resulted in greater SOC at these times than 1.5% LEA or the control. Soil OC for the 1.5% LEA + 1.5% WS and 3.0% LEA treatments decreased by 5.5 g kg^{-1} and 1.7 g kg^{-1} from 0 to 12 months, but were still 20% and 30% greater, respectively, than the control at this time. The majority of OC mineralization from amendments tended to occur by 3 months after application.

Table 2. Soil pH measured at 0–5, 5–15, and 15–30 cm depths 0 and 12 months after treatment application.

	Time (months)	
Treatment	0	12
	depth: 0–5 cm	
Control (+N,+P)	7.3b[†]	6.7
1.5% LEA[‡]	8.2a	6.8
3.0% LEA	8.3a	6.6
1.5% LEA+1.5% WS[§]	8.1a	6.9
p-value	<0.0001	0.25
	depth: 5–15 cm	
Control (+N,+P)	7.0b	6.6b
1.5% LEA	8.1a	6.9a
3.0% LEA	8.3a	6.9a
1.5% LEA+1.5% WS	8.2a	7.0a
p-value	<0.0001	0.013
	depth: 15–30 cm	
Control (+N,+P)	7.2b	6.5b
1.5% LEA	8.2a	6.8a
3.0% LEA	8.4a	7.0a
1.5% LEA+1.5% WS	8.0a	7.0a
p-value	0.0004	0.004

[†]Within time and depth, means followed by the same letter are not significantly different at $P < 0.05$.

[‡]LEA denotes lipid-extracted algae.

[§]WS denotes wheat straw.

Table 3. Soil electrical conductivity (EC; dS m^{-1}) at 0–5, 5–15, and 15–30 cm depths 0 and 12 months after treatment application.

	Time (months)	
Treatment	0	12
	depth: 0–5 cm	
Control (+N,+P)	0.3c[†]	0.3b
1.5% LEA[‡]	1.1b	1.9b
3.0% LEA	2.0a	4.3a
1.5% LEA+1.5% WS[§]	1.0b	1.0b
p-value	<0.0001	0.003
	depth: 5–15 cm	
Control (+N,+P)	0.4c	0.2b
1.5% LEA	1.2b	0.5b
3.0% LEA	1.9a	1.3a
1.5% LEA+1.5% WS	1.1b	0.5b
p-value	<0.0001	0.0002
	depth: 15–30 cm	
Control (+N,+P)	0.3d	0.2b
1.5% LEA	0.8b	0.5b
3.0% LEA	1.2a	1.2a
1.5% LEA+1.5% WS	0.5c	0.5b
p-value	<0.0001	0.0008

[†]Within time and depth, means followed by the same letter are not significantly different at $P < 0.05$.

[‡]LEA denotes lipid-extracted algae.

[§]WS denotes wheat straw.

Soil OC of LEA-amended bulk soil at the 15–30 cm depth tended to be less than at the 0–15 cm depth, especially at 0 months, but tended to become more similar with time (Figure 1). Observed treatment effects on SOC were generally similar for the two depths. Soil total N at the 0–15 cm depth 0 months after treatment application was significantly greater with 3.0% LEA (1944 mg kg^{-1}) compared to the control (1444 mg kg^{-1}) or 1.5% LEA (1649 mg kg^{-1}) but not 1.5% LEA + 1.5% WS (1850 mg kg^{-1}) (Figure 2a). By 3 months, total N at this depth was still greatest with 3.0% LEA (2063 mg kg^{-1}), with this trend generally continuing with time. Total N of all amended soils was greater than the control at 3 through 12 months. Values for soil total N were much more stable with time compared to SOC (Figure 1). No treatment differences occurred within the 15–30 cm depth for soil total N at 0 and 3 months after ammendment application (Figure 2b). By 6 months, however, trends were similar to that observed at 0–15 cm with 3.0% LEA-amendment increasing total N compared to the control.

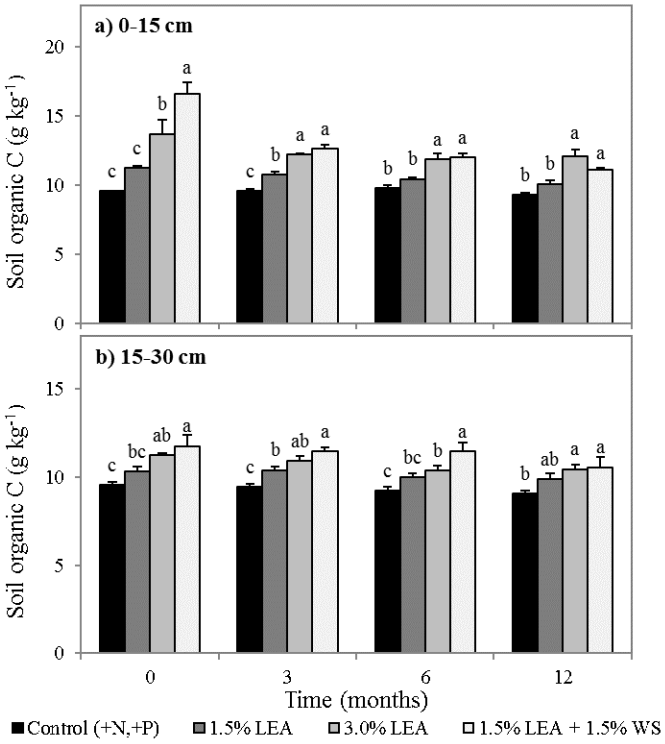

Figure 1. Soil organic C of bulk soil at a) 0–15 cm and b) 15–30 cm depths during the 12-month field incubation. Means within depth and time followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively. Bars above columns represent standard error of the mean.

3.3. Amendment Effects on Soil Aggregation and Carbon Dynamics within Aggregate Fractions

3.3.1. Aggregate formation

No treatment differences for aggregate MWD were noted within 0–5 and 15–30 cm depths at any sampling time ($P = 0.08$), except for 0–5 cm at 0 month ($P = 0.03$); however, differences ($P \leq 0.05$) were observed at 0, 6, and 12 months at the 5–15 cm depth.

The control resulted in greater MWD at 5–15 cm depth compared to 1.5 and 3.0% LEA-treated soil 0 months following treatment application (Figure 3). Six months after treatment application, MWD of the control was similar to that of soil amended with 3.0% LEA, but greater than the other two LEA treatments. Twelve months after treatment application, the 1.5 and 3.0% LEA treatments resulted in greater MWD than 1.5% LEA + 1.5% WS but not the control. Although an overall increase of MWD was observed over the 12-month study for the control as well as all organic amendments, 1.5 and 3.0% LEA-amended soil resulted in the greatest percentage increase in MWD (54% and 56%, respectively) compared to the control and 1.5% LEA + 1.5% WS treatment, which increased by 22% and 15%, respectively. Sodium contained in LEA (Table 1) may initially have decreased aggregate MWD following amendment application.

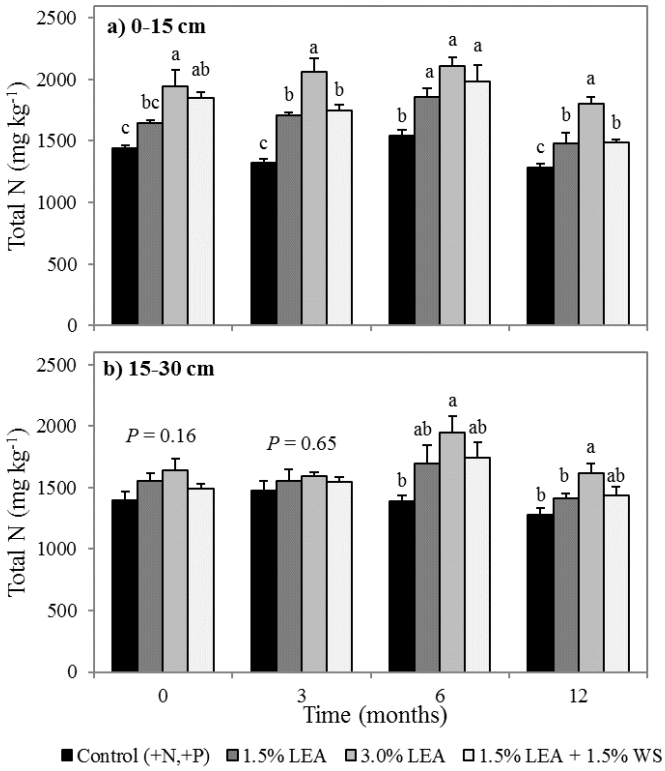

Figure 2. Total N of bulk soil at a) 0–15 and b) 15–30 cm depths over a 12-month field incubation. Mean total N within depth and time followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively. Bars above columns represent standard error of the mean.

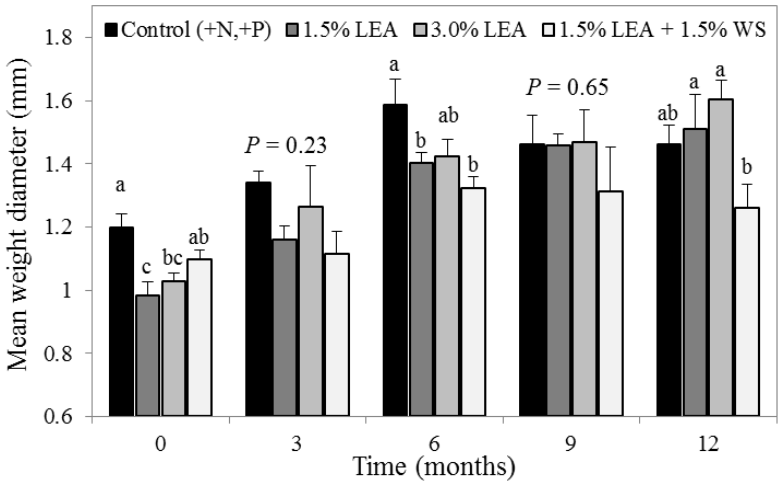

Figure 3. Aggregate formation represented as mean weight diameter over time within the 5–15 cm soil depth. Means followed by the same letter within time are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively. Bars above columns represent standard error of the mean.

3.3.2. Soil C and $\delta^{13}C$ dynamics

The natural abundances of the stable isotope $\delta^{13}C$ of Parrita soil was −16.3‰, while those for LEA and WS were −27.6‰ and −28.9‰, respectively. Since SOC relates closely to the plant material it originated from [22], the $\delta^{13}C$ value of Parrita soil is likely due to cultivation of C_4 grasses at this location. The natural difference between $\delta^{13}C$ values of soil and amendments allowed the determination of the relative contributions of C_3 LEA to SOC [24].

At 0–5 cm depth, significant differences in $\delta^{13}C$ were observed between the control and other treatments for all three fractions at each measurement time, except for the silt and clay fraction at 3 months (Figures 4a–c). At 0 months at this depth, macroaggregates from soil amended with 1.5% LEA + 1.5% WS or 3.0% LEA were more depleted in ^{13}C compared to the control (Figure 4a). At 3, 6, and 12 months, macroaggregates from all LEA treatments at 0–5 cm depth were more depleted in ^{13}C than the control and by 12 months, the 3.0% LEA treatment was most depleted in ^{13}C (−18.3‰).

Although microaggregate $\delta^{13}C$ trends over time at 0–5 cm were similar to those of macroaggregates, microaggregate signatures of all LEA amended soils were more ^{13}C depleted than control soil at each measurement time (Figure 4b). Also, different from macroaggregates, $\delta^{13}C$ signatures of microaggregates at 12 months for 3.0% LEA and 1.5% LEA + 1.5% WS were similar, but more negative and more like LEA's original signature ($\delta^{13}C$ = −27.6‰) than the control or 1.5% LEA treatment. At 0, 6, and 12 months at 0–5 cm depth, the silt and clay fraction of LEA-amended treatments also exhibited more negative $\delta^{13}C$ signatures than the control (Figure 4c). All aggregate fractions from soil amended with LEA exhibited more positive $\delta^{13}C$ values over time. The silt and clay fraction in soil from 0–5 cm tended to have the most negative signatures for LEA treatments at 0 months and the least negative at 12 months compared with macro- and microaggregates.

Differences in $\delta^{13}C$ signatures were also observed at 5–15 cm for macroaggregates, microaggregates, and the silt and clay fraction at all sampling times, except again for the silt and clay fraction at 3 months (Figures 4d,e,f). Macroaggregates from LEA-amended soils at 5–15 cm were more depleted in ^{13}C at each measurement time compared to the control (Figure 4d). Values for soils receiving of 3.0% LEA and 1.5% LEA + 1.5% WS were also more depleted than 1.5% LEA. Microaggregate $\delta^{13}C$ of soil amended with 3.0% LEA and 1.5% LEA + 1.5% WS were most negative and similar at 0, 3, and 6 months. By 12 months, 1.5% LEA + 1.5% WS was most depleted (Figure 4e). Microaggregates from all LEA treatments were more depleted in ^{13}C compared to the control at all sampling times. For the 3.0% LEA treatments at 5–15 cm depth, $\delta^{13}C$ values of microaggregates tended to be slightly more depleted (−18.8‰) compared to macroaggregates (−18.3‰) (Figures 4d,e). Silt and clay $\delta^{13}C$ signatures over time at 5–15 cm generally followed trends similar to those at 0–5 cm (Figures 4c,f).

While differences in $\delta^{13}C$ were noted between the control and LEA-amended soil at 15–30 cm for all size fractions and measurement times, signatures tended to be less depleted in ^{13}C at this depth compared to 0–5 and 5–15 cm regardless of LEA-treatment (Figures 4g,h,i), likely because the bottom 10 cm of all columns contained only unamended Parrita soil. $\delta^{13}C$ values of macroaggregates and free silt and clay fractions for 1.5% LEA and 3.0% LEA treated soils were similar 12 months after treatment imposition at 15–30 cm depth; however, free microaggregate signatures for 3.0% LEA and 1.5% LEA + 1.5% WS treatments were most depleted in ^{13}C at this time (Figure 4h). Unlike signatures at 0–5 and 5–15 cm for 3.0% LEA-amended soil, $\delta^{13}C$ at 15–30 cm tended to be slightly more depleted for macroaggregates (−17.3‰) than for free microaggregates (−17.1‰) or silt and clay (−16.4‰) (Figure 4).

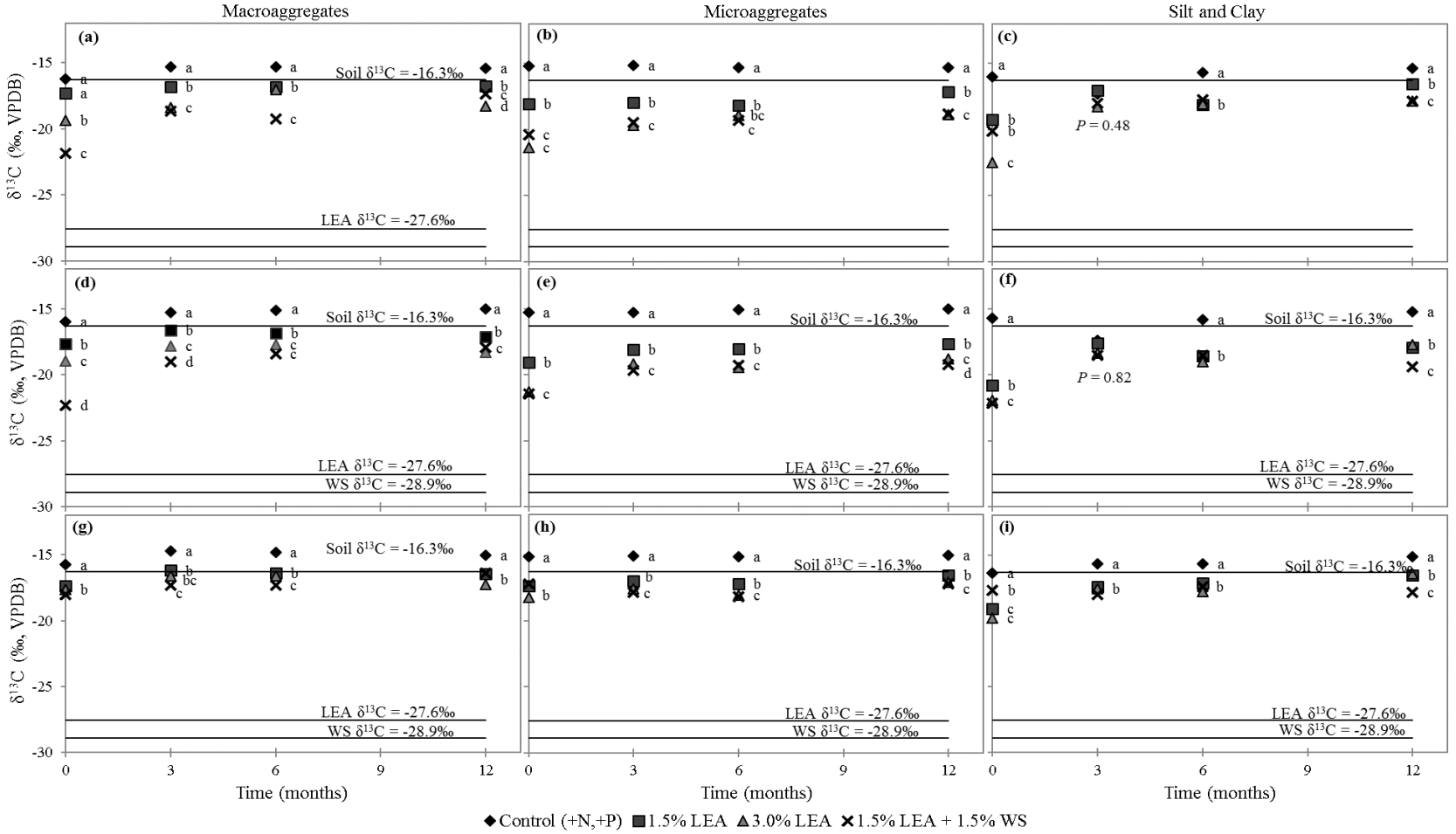

Figure 4. Fallow soil $\delta^{13}C$ (‰) measured over a 12-month field incubation at depths (rows) of 0–5 cm (a-c), 5–15 cm (d-f), and 15–30 cm (g-i) and aggregate size fractions (columns) of macroaggregates (a,d,g), microaggregates (b,e,h), and silt and clay (c,f,i). Means followed by the same letter within depth, size fraction, and time are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively.

Soil OC in all aggregate size fractions from the 0–5 cm depth tended to decrease with time for both LEA-amended soil and the control (Figures 5a,b,c). Soil macroaggregate OC was significantly different between the control and that of at least one LEA treatment at 0, 3, and 6 months (Figure 5a). By 12 months, however, no differences were observed (P = 0.099). Soil amended with 1.5% LEA + 1.5% WS generally resulted in the greatest SOC. Significant SOC differences for microaggregates within the 0–5 cm depth were observed at 0 and 12 months after treatment application but only at 12 months for the silt and clay fraction (Figures 5b,c). Microaggregate SOC tended to be greatest for 3.0% LEA and 1.5% LEA + 1.5% WS (Figure 5b). Soil OC in the silt and clay fraction at 12 months was again greater for the 1.5% LEA + 1.5% WS and 3.0% LEA treatments.

At 5–15 cm depth, SOC differences were noted within both macro- and microaggregates fractions at each sampling time (Figures 5d,e). Differences in SOC for silt and clay fraction, however, were observed only at 6 and 12 months (Figure 5f). Macro- and microaggregate-associated SOC at this depth at 12 months was greater for 1.5% LEA (1.4% and 1.2%, respectively), 3.0% LEA (1.6% and 1.4%, respectively), and 1.5% LEA + 1.5% WS (1.5% and 1.4%, respectively) compared to the control (1.2% and 1.1%, respectively) (Figures 5d,e). Soil OC of the silt and clay fraction at 12 months was also greater in soil amended with 3.0% LEA (2.8%) or 1.5% LEA + 1.5% WS (3.0%) compared to the control (2.4%; Figure 5f). Soil OC in both macro- and microaggregates tended to decrease with time, but remained relatively more stable in the silt and clay fraction.

Differences in macroaggregate SOC from 15–30 cm depth were observed at 0, 6, and 12 months and in microaggregates at 0, 3, and 12 months, while that in the silt and clay fraction was different at 6 and 12 months (Figures 5g,h,i). Macroaggregate SOC for all LEA treatments at 12 months was greater than the control (Figure 5g). Soil amended with 1.5% LEA + 1.5% WS resulted in the greatest microaggregate SOC concentration at 12 months, followed by 3.0% LEA (Figure 5h). Similarly, 1.5% LEA + 1.5% WS also resulted in the greatest SOC in the silt and clay fraction (Figure 5i). Overall, the general trends observed for SOC in aggregate size fractions at 15–30 cm depth were similar to those of the 0–5 and 5–15 cm depths (Figure 5).

LEA-C from the 1.5% LEA treatment as a percentage of total SOC in aggregate-size fractions at both 0–5 and 5–15 cm was generally less in the macroaggregate compared to the microaggregate and silt and clay size fractions throughout most of the incubation period (Figures 6a-c). Approximately 42% of OC added with the 1.5% LEA treatment remained in the 0–15 cm depth 12 months after amendment application. Differences were also detected at all sampling times and depths for the percentage of C within size fractions derived from 3.0% LEA addition except in the 15–30 cm depth and at 12 months (Figures 6d–f). Trends for LEA-C storage within aggregate fractions with this treatment were similar to those with 1.5% LEA, but percentages derived from 3.0% LEA addition were greater. Approximately 66% of LEA-C remained in the 0–15 cm depth 12 months after adding 3.0% LEA. Differences were not observed 0 months after the 1.5% LEA + 1.5% WS addition in any depth and 3 months after application in the two deeper depths (Figures 6g–i). Greater microaggregate-C remained 12 months after adding the 1.5% LEA + 1.5% WS treatment compared to macroaggregate-C in any depth.

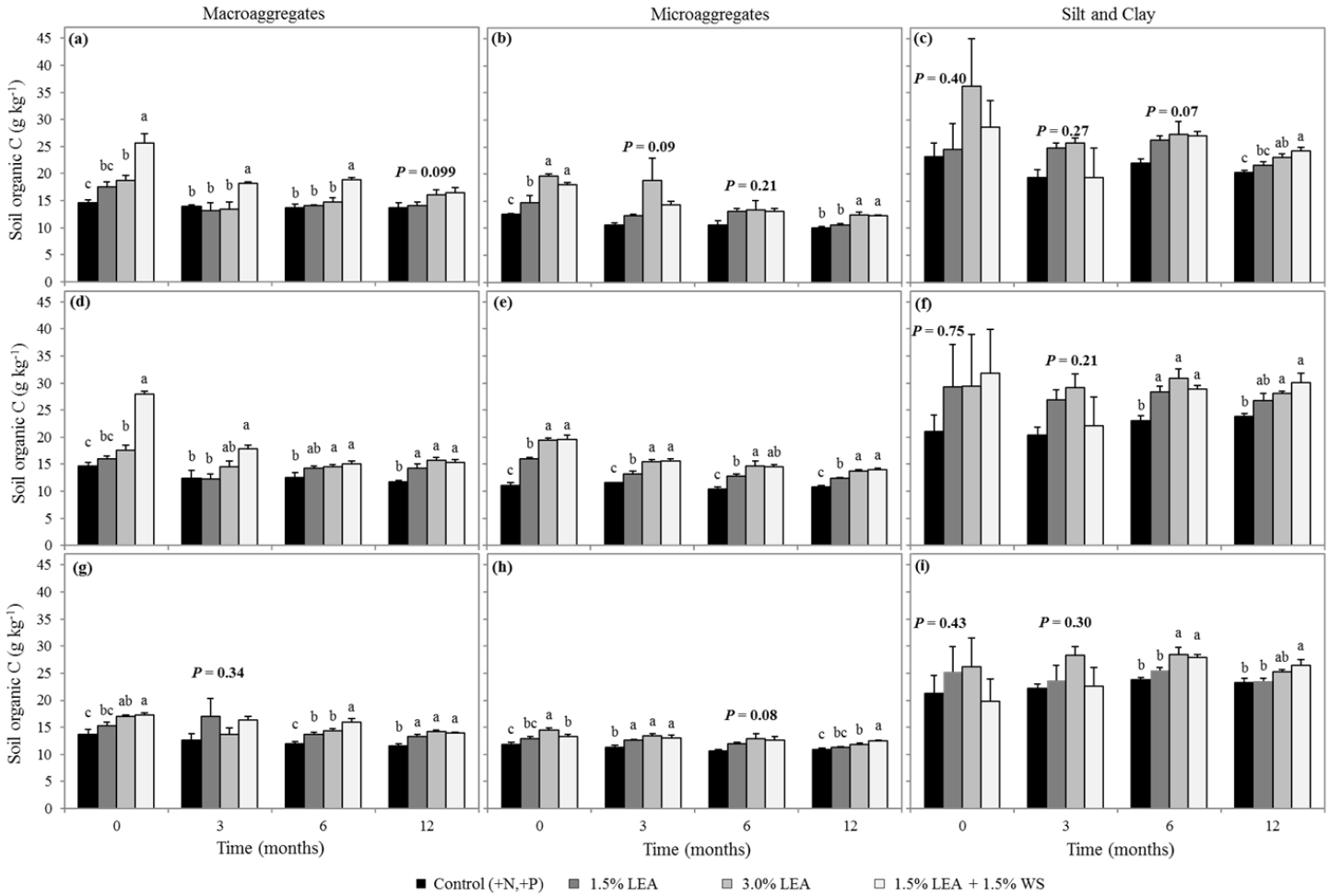

Figure 5. Soil organic C measured over a 12-month field incubation at depths (rows) of 0–5 cm (a-c), 5–15 cm (d-f), and 15–30 cm (g-i) and size fractions (columns) of macroaggregates (a,d,g), microaggregates (b,e,h), and silt and clay (c,f,i). Means followed by the same letter within depth, size fraction, and time are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively.

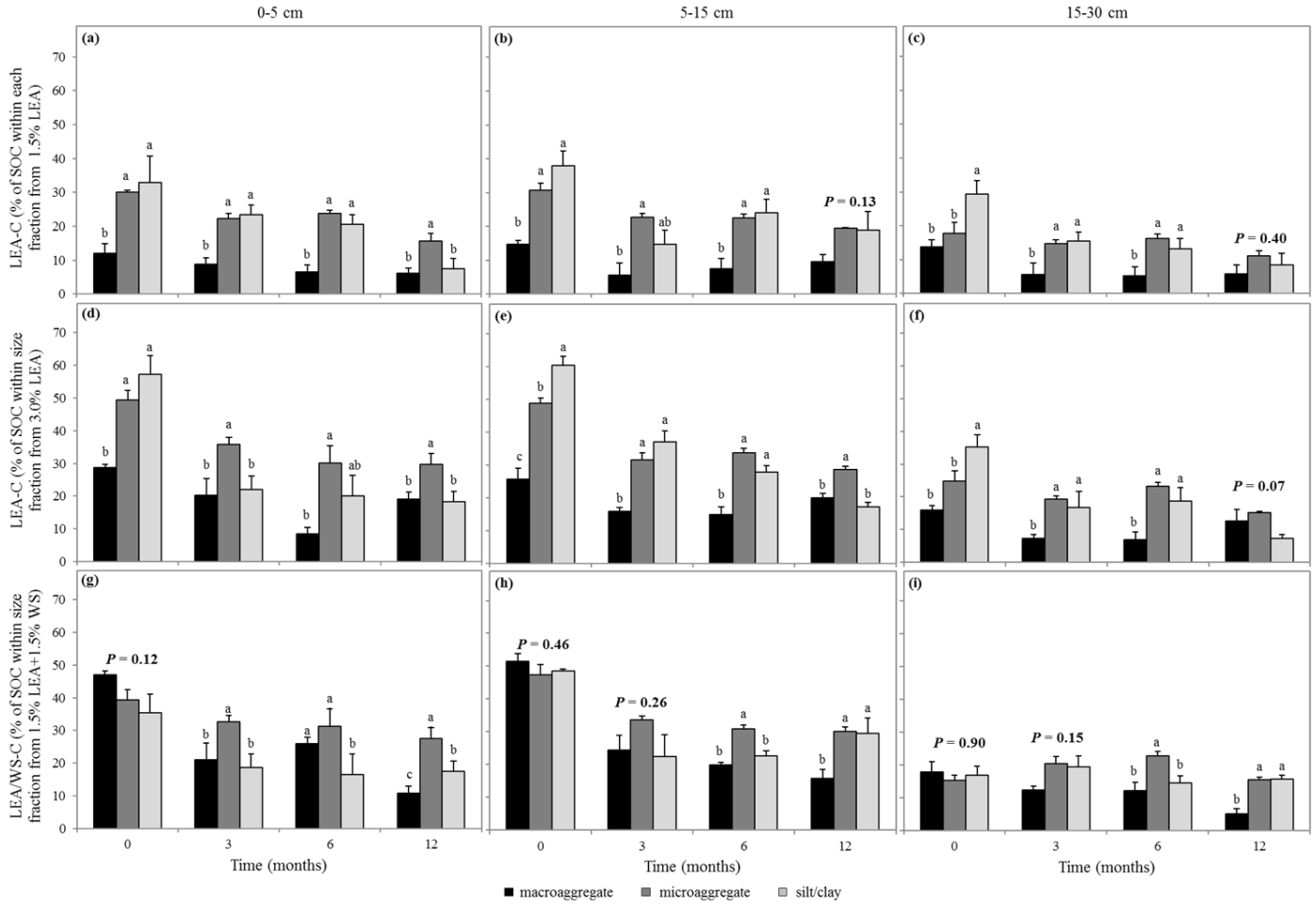

Figure 6. Percentage of organic C at depths (columns) of 0–5 (a, d and g), 5–15 cm (b, e and h), 15–30 cm (c, f and i) and macroaggregate, microaggregate, and silt and clay (>250, 53–250, and <53 µm, respectively) size fractions derived from treatments (rows) of 1.5% LEA (a-c), 3.0% LEA (d-f), and 1.5% LEA + 1.5% WS (g-i). Means within depth, treatment, and time followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA denotes lipid-extracted algae and WS denots wheat straw. Bars represent standard error of the mean.

4. Discussion

Greater aggregate MWD for the control compared to LEA treatments was observed from 0 to 6 months after treatment application, possibly resulting from aggregate disruption when soil was collected, homogenized, and mixed with treatments [43], or elevated levels of Na^+ in LEA and K^+ in WS may also have negated the positive effects of organic amendments on aggregation. Whalen and Chang [44] attributed the decrease of macroaggregates after manure application to the dispersion of soil colloids caused by monovalent cations of Na^+ and K^+ in the manure. However, MWD 12 months after 3.0% LEA application tended to be greater compared to the control or other treatments, followed by 1.5% LEA addition. Greater MWD in soil amended with 3.0% LEA indicated a greater proportion of macro- and microaggregates, and potentially greater SOC storage over time [5,6]. Chivenge et al. [43] also observed increased MWD over time, which was determined to be the result of a greater proportion of macro- and microaggregates.

Soil treated with 1.5 or 3.0% LEA enhanced aggregate formation compared to 1.5% LEA + 1.5% WS. The addition of organic amendments and plant residues, such as animal manure and WS, respectively, have been demonstrated to improve soil stability by enhancing aggregate formation [9,44]; however, our study indicated that LEA applied with WS had less effect on aggregate formation compared to LEA alone, possibly because of the initial more rapid mineralization, but ultimately greater proportion of recalcitrant compounds in LEA compared to WS [14]. Quickly decomposing organic materials with a narrow C:N ratio may produce a rapid but likely only temporary increase in aggregate production, whereas slowly decomposing or more stable organic materials may produce a lesser but more permanent improvement in aggregation [45,46]. Thus, the combination of both quickly mineralizable and recalcitrant portions (algaenans) in LEA, may have enhanced both aggregate formation and stability. After 6 months, the concentration of soil total N was nearly as great at 15–30 cm as at 0–15 cm, implying significant movement of LEA associated inorganic N and dissolved organic N (DON). Lynch et al. [47] recovered greater than 80% of composted manure-N in coarser soil fractions one year after application.

Macroaggregate $\delta^{13}C$ from soil amended with 3.0% LEA was the most ^{13}C depleted treatment 12 months after application, indicating greater macroaggregate associated LEA-C for this treatment at 0–5 and 5–15 cm soil depths (Figures 4a,d). Microaggregates from soils receiving any LEA treatment stored more C compared to the control to 15 cm depth as evidenced by more depleted $\delta^{13}C$ values for these treatments (Figures 4b,e). $\delta^{13}C$ signatures to 15 cm depth at 12 months also indicated greater LEA-C in micro- compared to macroaggregates. However, at 15–30 cm depth, there was a tendency for greater LEA-C in macro- rather than microaggregates. These results indicated transformation of LEA during aggregate formation since $\delta^{13}C$ values increased from 0 to 12 months.

Based on $\delta^{13}C$ results, greater LEA-C was initially associated with the silt and clay fraction when LEA was applied alone, but over time, greater LEA-C storage was observed in the macro- and microaggregate fractions, implying that aggregate formation was enhanced with LEA application. Compared to the LEA plus WS treatment, LEA applied alone generally was found more quickly in the microaggregate and silt and clay fractions. These results agree with Zhang et al. [48], who reported increased glucose-derived ^{13}C in silt and clay fractions and which was greater than in macroaggregates and microaggregates on day 30 of incubation in all test treatments. Microaggregate associated C may be more physically protected and biochemically more recalcitrant than that of

macroaggregates [9,10], and therefore, it may be possible to sequester more SOC with LEA-amendment.

The change of $\delta^{13}C$ isotope composition from 0 to 12 months may be the result of both: (1) preferential utilization or decomposition of substances with ^{12}C versus ^{13}C, and (2) stabilization of organic materials after passing one or more microbial utilization cycles, which releases ^{13}C-depleted CO_2 compared to OM in C4 soils typical of arid and semi-arid environments [49]. With the above two scenarios occurring together and organic material likely being more protected in macro- and microaggregate fractions, the difference of $\delta^{13}C$ from 0 to 12 months for 1.5 and 3.0% LEA was greater in the silt and clay fraction compared to macro- and microaggregates. Likely, LEA-C in all fractions was being utilized by microorganisms, therefore increasing $\delta^{13}C$, but it was only in macro- and microaggregates that the more recalcitrant C, which is more depleted in ^{13}C, was being stored [43].

Soil OC tended to be greater in the silt and clay fraction compared to macro- or microaggregates, possibly resulting from aggregate destruction during soil collection and treatment preparation, consequently exposing protected OM to decomposers and accelerated SOM decomposition [50]. Parrita soil was homogenized by mixing prior to treatment preparation for the control and all other treatments in order to reduce experimental error, but consequently may have somewhat altered soil structure [43]. Comparing the 0–5 and 5–15 cm depths 12 months after treatment application, macroaggregate SOC tended to be greater at 0–5 cm, while microaggregate SOC was greater at 5–15 cm. Soil amended with 1.5% LEA, 3.0% LEA or 1.5% LEA + 1.5% WS resulted in greater SOC after 12 months for all soil size fractions and depths. Thus, LEA addition increased SOC at least in the short-term. Chivenge et al. [43] observed greater macro- and microaggregate SOC and N than in the silt and clay fraction. Bhattacharyya et al. [51] reported that the distribution of newly added manure C in aggregates increased with aggregate size. Udom et al. [52] reported SOC to be more enriched in larger aggregates than smaller aggregates in soil amended with poultry manure and spent mushroom waste.

Lipid-extracted algae residue is mineralizable, but LEA tends to be more resistant to decay than WS [14], and therefore, potentially may store greater SOC. Yousaf et al. [53] reported increased SOC and reduced C-mineralization in biochar-amended soil compared to conventional biowaste amendments possibly due to the presence of recalcitrant biochar-C. Recalcitrance of LEA is likely associated with nonhydrolyzable macromolecules located in microalgae cell walls that comprised 13.4% (DM basis) of lipid-extracted *Nannochloropsis salina* algae residue.

5. Conclusions

This research demonstrated the potential beneficial use of LEA as a soil amendment to improve soil structure through aggregate development and enhanced SOC storage. Soil application of LEA enhanced initial aggregate formation and SOC storage by increasing aggregate MWD and macro- and microaggregate associated SOC over time. Lipid-extracted algae was initially more recalcitrant than WS and might contribute to longer-term SOC storage. However, caution should be taken to reduce possible salinity-associated effects of over-applying LEA, such as hindering plant growth and soil degradation from excess Na^+. Lipid-extracted algae, when applied in proper amounts, may not only supply plant essential nutrients and improve soil quality, but should also enhance the economic and environmental viability of algae-produced biofuel.

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Conflict of interest

All authors declare no conflicts of interest in this paper.

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