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Assessment of Soil Aggradation through Soil Aggregation and Particulate Organic Matter by Riparian Switchgrass Buffers

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Abstract: The restoration of riparian zones has been an important issue in many regions for the recovery of ecosystem functions. The objective of this study was to assess soil aggradation in a 7-year established riparian switchgrass buffer (SGB) and in a non-buffered riparian zone with an annual row crop (ARC). We measured the aggregate size distribution and stability of macroaggregates, aggregate-associated soil organic carbon, soil organic matter fractions and the chemical composition of light particulate organic matter to monitor soil aggregation in a riparian soil following the conversion of agricultural row crops to switchgrass filters. Aggregate size fractions were separated by wet sieving using the aggregate size-stability protocol. The proportion of soil and total organic C was quantified for each aggregate size class. Soil organic matter fractions were isolated by size and density into light particulate organic matter and heavy particulate organic matter and mineral fraction organic matter. The categorization of aggregates by size and water stability (slaking resistance) showed a significantly larger ($p < 0.001$) proportion of water-unstable large macroaggregates ($>2000 \mu\text{m}$) under SGB (34%) compared to that under ARC (29%), while the proportion of water-unstable small macroaggregates ($250\text{--}2000 \mu\text{m}$) was significantly higher under ARC (14%) than under SGB (10%). Our results showed that the amounts of light and heavy particulate organic matter did not change in the short-term (7 years) after SGB establishment. It appears that the lower soil stabilization and soil organic C storage under SGB is related to (i) the large number of coarse roots; (ii) lower inputs of light and heavy particulate organic matter; (iii) no changes in the alkyl-C/O-alkyl-C ratio over time; and (iv) light particulate organic matter with a high C/N ratio.

Keywords: riparian zone; switchgrass buffer; soil aggregation; organic matter fractions

1. Introduction

Riparian zones have important geomorphic and hydrologic roles and support high levels of biological productivity. They have a disproportionate ecological role in the landscape considering their narrow extent, which makes them a good example of small natural features. Characteristically, riparian zones increase species richness in the landscape and provide key services to society, such as soil fertility, water purification, and recreation [1]. Healthy riparian areas stabilize stream channels, provide sediment storage, serve as nutrient sinks for the surrounding watershed, and improve the quality of water leaving the watershed [2]. Most riparian zones in agricultural land have long been

negatively influenced by human activities. The frequent disturbance of riparian zones has a direct influence on water quality, hydraulic alteration of waterways, reduction in soil quality, and disruption of wildlife habitats and populations [3]. Reforestation of riparian zones is increasingly practiced in many regions for purposes of biodiversity conservation, bank stabilization, and improvement in water quality. This occurs in spite of the actual benefits of reforestation for recovering underlying soil properties, and its functions remain poorly understood [4].

Considerable attention has recently been focused on the restoration of riparian forest and grass buffer systems. Numerous approaches have been adopted to mitigate the adverse impacts of agricultural practices within the context of a bio-assimilative strategy. These include the restoration of riparian vegetative buffers [5]. Most restoration programs include cool-season (C3) and warm-season (C4) grasses to reduce water runoff and sediment loss and help soil development processes by improving soil organic matter, soil structure, and soil water- and nutrient-holding capacity [6–8]. In 1990, the Agroecology Issues Team of the Leopold Center for Sustainable Agriculture at Iowa State University (ISU) established a multi-species riparian buffer along Bear Creek in central Iowa, USA. Bear Creek is typical of many streams in central Iowa, where the primary land use within the watershed, including the riparian zone, is row crop agriculture (corn and soybean) or intensive grazing [3,9]. Four years after the establishment of the multi-species riparian buffer along Bear Creek, Schultz et al. [9] reported dramatic alterations in the appearance and function of the riparian buffer. Specifically, the root biomass increased significantly below the multi-species riparian buffer compared with agricultural crops. Also, nitrate-nitrogen concentrations in the multi-species riparian buffer never exceeded 2 mg L^{-1} , whereas the average levels in the adjacent agricultural fields exceeded 12 mg L^{-1} . These results support the idea that the new aggrading conditions led to the restoration of the multi-species riparian buffer along Bear Creek. In aggrading systems, organic inputs lead to the formation and stabilization of aggregates, which in turn can protect soil organic matter from decomposition, leading to further aggregate stabilization [10]. However, in aggrading systems, plant species can differ in their ability to influence soil aggregation and improve soil productivity by carbon sequestration. For example, Jastrow [11] concluded that an increase in root production and root biomass under C4 prairie graminoids might confer a sore advantage over introducing C3 Eurasian grasses for the development of water-stable aggregates. Switchgrass may improve soil quality by sequestering C in the switchgrass-soil ecosystem owing to its high biomass [12] and deep root system [13]. However, Scott [14] reported that C4 grasses did not affect aggregate-size distribution or organic matter concentration in spite of twofold differences in root biomass and threefold differences in N cycling compared to C3 grasses. Similarly, Corre et al. [15] concluded that the slow accumulation of C4-derived soil organic carbon is an important consideration for its use in restoring riparian and conservation areas. Indeed, Franzluebbers et al. [16] reported that the storage of soil organic carbon occurred at a rate of $100 \text{ g m}^{-2} \text{ y}^{-1}$ during the first 10 years of establishment under grazed tall fescue (C3) and at a rate of $33 \text{ g m}^{-2} \text{ y}^{-1}$ under hayed bermudagrass (C4). Ma et al. [13] also concluded that several years of switchgrass culture would be required to realize a soil C sequestration benefit. However, previous studies have shown that variation in the estimated rates of carbon accrual can be attributed to a variety of factors including soil texture, plant community, biomass production, and species richness. Soil C and N have also been shown to increase with species richness, as species richness has been shown to promote plant biomass production [17]. On the other hand, plant species differ in biomass production, and soil C storage is primarily controlled by two fundamental processes: net primary production (NPP) and decomposition. An increase in NPP leads to an increase in C storage, whereas increased decomposition has the opposite effect [18].

In aggrading systems where particulate organic matter (POM) is continually being added to the soil, the destruction of macroaggregates due to the decomposition of POM is offset by the continual input of new POM. Therefore, as the stability of a portion of the macroaggregates decreases due to decomposition processes, other macroaggregates form and become stabilized, and, moreover, the stabilization of new macroaggregates may occur at the expense of the breakdown of older, less

stable macroaggregates [19]. The aggregation process itself is a means to both conserve organic matter and allow the stored organic matter to function as a reservoir of plant nutrients and energy. Also, the role of organic matter in aggregation has major implications for the functioning of soil in regulating air and water infiltration [20]. This interaction between soil organic matter and soil aggregates has allowed the development of indicators associated with the process of soil aggregate dynamics and organic matter associated with aggregates [21]. The purpose of this study was to evaluate the process of soil aggradation following the conversion of a riparian agricultural row crop system to a riparian switchgrass filter. The specific objectives were to: (i) study soil aggregate size distribution and stability of macroaggregates, (ii) study aggregate-associated soil organic carbon, (iii) quantify the various fractions of soil organic matter, and (iv) determine the chemical composition of the light particulate organic matter fraction.

2. Results

2.1. Aggregate Size Distribution and Stability of Macroaggregates

Analysis of water-stable aggregates showed that the proportion of soil present as water-stable large macroaggregates ($>2000\ \mu\text{m}$) changed no more under SGB (4.65%) than it did under ARC (3.99%) in the short-term (7 years) after switchgrass establishment. The proportion of soil aggregates present as water-unstable large macroaggregates ($>2000\ \mu\text{m}$) was greater ($p < 0.001$) under SGB (34.0%) than under ARC (29.3%). In contrast, the proportion of soil aggregates present as water-unstable small macroaggregates ($250\text{--}2000\ \mu\text{m}$) was significantly larger ($p < 0.001$) under ARC (14.20%) than under SGB (9.8%). Furthermore, the proportion of soil present as water-stable small macroaggregates ($250\text{--}2000\ \mu\text{m}$), microaggregates ($53\text{--}250\ \mu\text{m}$), and silt- plus clay-size particles ($<53\text{-}\mu\text{m}$) showed no significant differences ($p > 0.05$) under SGB compared to under ARC, see Table 1.

The ratio of large water-stable macroaggregates to large water-unstable was 0.16 under SGB and 0.14 under ARC, while the ratio of small water-stable macroaggregates was 2.27 under SGB and 1.57 under ARC.

Table 1. ANOVA results from the comparison of the proportional of water-stable and water-unstable aggregates in each aggregate fraction from switchgrass buffer (SGB) and non-buffered annual row crop (ARC).

Variable	ANOVA p Value	Mean SGB	Mean ARC	Standard Error	Contrast SGB vs. ARC
Water-stable aggregates (%)					
$>2000\ \mu\text{m}$	0.001	5.59	3.99	0.99	SGB > ARC
$250\text{--}2000\ \mu\text{m}$	0.873	22.29	22.30	1.57	NS
$53\text{--}250\ \mu\text{m}$	0.052	13.26	14.20	1.79	NS
$<53\ \mu\text{m}$	0.460	14.25	16.00	1.13	NS
Water-unstable aggregates (%)					
$>2000\ \mu\text{m}$	0.001	33.95	29.30	2.10	SGB > ARC
$250\text{--}2000\ \mu\text{m}$	0.001	9.80	14.20	1.09	ARC > SGB

Bold text indicates significance at the 0.05 level, NS indicates not significant if $p > 0.05$. Aggregates that survive slaking are labeled as stable and those that do not survive are labeled as unstable.

2.2. Associated Organic Carbon to Soil Aggregate Fractions

The concentration of organic C associated with large and small water-stable macroaggregates was $0.47\ \text{mg C g}^{-1}$ greater under SGB than that under ARC. However they were not significantly different ($p > 0.05$) (Table 2). For water-stable microaggregates and silt-plus clay-size particles, the concentrations of organic carbon C were not significantly different ($p > 0.05$) for SGB ($5.16\ \text{mg C g}^{-1}$) compared to ARC ($5.10\ \text{mg C g}^{-1}$). The total concentration of organic C associated with water-unstable large and small macroaggregates did not exhibit significant for change under SGB and ARC at levels of $9.81\ \text{mg C g}^{-1}$ and $9.34\ \text{mg C g}^{-1}$, respectively, see Table 2.

Table 2. ANOVA results from comparison of the concentration of carbon associated with water-stable and water-unstable aggregates in each aggregate size fraction from switchgrass buffer (SGB) and non-buffered annual row crop (ARC).

Variable	ANOVA <i>p</i> Value	Mean SGB	Mean ARC	Standard Error	Contrast SGB vs. ARC
Carbon associated with water-stable aggregates/mg C g ⁻¹					
>2000 µm	0.054	1.40	1.30	0.36	NS
250–2000 µm	0.060	6.37	6.00	0.99	NS
53–250 µm	0.071	2.59	2.70	0.57	NS
<53 µm	0.086	2.57	2.40	0.54	NS
Carbon associated with water-unstable aggregates/mg C g ⁻¹					
>2000 µm	0.070	6.70	6.50	0.93	NS
250–2000 µm	0.057	3.11	2.84	0.75	NS

NS, not significant if $p > 0.05$. Aggregates that survive slaking are labeled as stable and those that do not survive are labeled as unstable.

2.3. Organic Carbon Fractions

Total soil organic carbon in the SGB plots was not significantly different (21.61 mg C g⁻¹) than that under the ARC (20.81 mg C g⁻¹). The carbon content associated with the mineral fraction had the same pattern as the total soil organic carbon in the whole soil (Table 3). No significant differences in the concentration of The light particulate organic matter (LPOM-C) were observed between SGB (1.52 mg C g⁻¹) and ARC (1.36 mg C g⁻¹). The amount of total particulate organic matter (TPOM-C) under SGB and ARC systems accounted for 7.0% and 6.5%, respectively, of the total organic C. In addition, no significant differences were observed between SGB and ARC systems in the and heavy particulate organic matter (HPOM-C) (Table 3). Our results reveal that the 7-year-old switchgrass gained 0.81 mg C g⁻¹ of total organic carbon, but this amount was not significantly different from the total amount of organic carbon under the cropped system.

Table 3. ANOVA results from a comparison of the concentration of carbon associated with soil organic matter fractions from switchgrass buffer (SGB) and non-buffered annual row crop (ARC).

Variable	ANOVA <i>p</i> Value	Mean SGB	Mean ARC	Standard Error	Contrast SGB vs. ARC
TSOC	0.064	21.61	20.81	2.22	NS
MAC	0.126	19.75	19.09	2.05	NS
LPOM-C	0.264	1.52	1.36	0.70	NS
HPOM-C	0.062	0.34	0.30	0.12	NS
TPOM-C	0.165	1.86	1.66	0.71	NS

TSOC, total soil organic carbon; MAC, mineral-associated carbon; LPOM-C, carbon associated with light particulate organic matter; HPOM-C, carbon associated with heavy particulate organic matter; TPOM-C, total particulate organic matter. NS, not significant if $p > 0.005$.

2.4. Chemical Composition of LPOM

Table 4 shows the relative NMR signal intensity for different types of carbon in the light particulate organic matter (<1.85 g cm⁻³) at a depth of 0–15 cm under SGB and non-buffered ARC fields. The major signal obtained by the fractionation procedures described above was from the 46–110 ppm region (O-alkyl carbon) with peaks at 74 ppm, indicating that carbohydrates were quantitatively the most significant compounds in this organic matter fraction. These spectra accounted for 52–59% of the total area under the NMR spectra for samples from the ARC system during July and November, and 64–59% under the SGB, respectively. Carbohydrates comprise 50–70% of dry plant tissues and thus are the most abundant materials added to soil in the form of plant residue [22]. The contribution of alkyl carbon accounted for 46% of the total area under NMR spectra during July and decreased to 28% in November. On the other hand, under SGB between July and November, no changes were observed, with the levels remaining between 31–32%. The carbonyl carbon occurred in the lowest quantities (2–6%). The result

from the NMR analysis confirms the hypothesis that the light particulate organic matter-C is a fraction that is composed of partially decomposed organic matter. Changes in the ratio of O-alkyl to alkyl-C over time have been used to understand decomposition processes [23]. The ratio of O-alkyl to alkyl carbon was lower in July (0.48) and increased in November (0.90) under the ARC system. The lower ratio during July is associated with a higher decomposition rate that is associated with the loss of the most easily metabolized carbohydrates and the accumulation of alkyl-C [24]. In November, the amount of carbon in the alkyl structure decreases, reflecting an increase in the amount of carbon in the O-alkyl structure. However, the results suggest a slower decomposition of O-alkyl carbon in the SGB soils compared with the ARC system soils, because the ratio of O-alkyl to alkyl C does not change over time in July (0.50) and increases in November (0.55). The lower decomposition rate observed under switchgrass suggests that the presence of lignin, which is much more resistant to decomposition, plays an important role in the chemical composition of the light particulate organic matter-C under the SGB. Lignin and lignin-N contents are primary factors controlling the rate of organic matter decomposition in terrestrial ecosystems [24]. Akin [25] found lignin values twice as high in C4 grasses compared C3 grasses (8.4 vs. 4.3, respectively). Consistent with these results, we found a higher C/N light particulate organic matter-C ratio under SGB (18–20) than under the ARC system (14–15).

Table 4. The relative proportion of different types of carbon in the light particulate organic matter (LPOM-C) at a depth of 0–15 cm under switchgrass buffer and non-buffered annual row crop. The chemical composition of LPOM samples were examined with a solid-state ^{13}C nuclear magnetic resonance spectrometer.

Signal Intensity/% of Total Intensity	Switchgrass Buffer		Annual Row Crop	
	July	November	July	November
Alkyl	32	31	46	28
O-Alkyl	64	59	52	59
Aromatic	3.0	7.5	15	17
Carbonyl	2	2	11	7

3. Discussion

Weaver et al. [26] found that the roots of warm-season prairie grasses (C4) are coarser, longer-lived, and more resistant to decay than the roots of cool season grass (C3). Also, Hetrick et al. [27] and Hetrick et al. [28] observed that warm season grasses (C4) have coarser root systems with fewer primary roots of larger diameters. Tufekcioglu et al. [29], working in the same research area used in this study, reported that switchgrass (C4) had significantly higher amounts (1248 kg ha^{-1}) of dead fine roots (0–2 mm) in the top 0–35 cm of soil compared to that found under cropped fields (650 kg ha^{-1}), but lower amounts than that found under cool-season grass (C3) (2225 kg ha^{-1}). In contrast, the amount of live small root biomass (2–5 mm) was highest under switchgrass (1861 kg ha^{-1}) compared with that under cool-season grass (393 kg ha^{-1}) and that under cropped systems (207 kg ha^{-1}). However, the dead small roots biomass (2–5 mm) was significantly greater under cool season grass (C3). Pickle [30], also working in the same experimental area used in this study, reported that 7 years after the establishment of the switchgrass (C4) filter, the amount of biomass-C was $161 \text{ mg C kg soil}^{-1}$ compared with $100 \text{ mg C kg soil}^{-1}$ under the cropped system. In both systems, the amount of biomass-C was significantly lower than that found under cool-season grass (C3) ($327 \text{ mg C kg soil}^{-1}$). Scott [1] observed that a threefold difference in root biomass among the grass species did not alter the proportion of macroaggregates or the amount of C associated with aggregates 10 years after the establishment of Fergus Falls grass (C4). Franzluebbers et al. [16] reported that soil organic matter and total N, to a depth of 200 mm, was accumulated at greater rates under grazed tall fescue (C3) than under bermudagrass (C4), and that storage of soil organic C occurred at a rate of $100 \text{ g m}^{-2} \text{ year}^{-1}$ during the first 10 years of establishment under fescue (C3) grasses and at a rate of $33 \text{ g m}^{-2} \text{ year}^{-1}$ under grazed bermudagrass (C4). Ma et al. [13] reported that management practices such as N

application, row spacing, and harvest frequency did not alter soil C concentrations in the 2 to 3 years following the establishment of switchgrass; however, in the long-term, i.e., 10 years after establishment, the switchgrass had sequestered more soil C than an adjacent fallow soil. Ma et al. [13] concluded that switchgrass would accumulate soil C, but it may take several years before any increases are detectable. Corre et al. [15] concluded that it took 16 to 18 years after planting for the total SOC under the C4 grass to approach a level similar to that under the original C3 grass.

Our results show that 7 years after the establishment of switchgrass, the new aggregate formation consisted mainly of unstable large macroaggregates that resulted from the aggregation of smaller fractions. Several mechanisms can support our results: (i) coarse roots [27,28]; (ii) longer-lived and more resistant to decay roots [26]; (iii) lower biomass-C [28], (iv) lower rates of mineralization [31]; (v) lower amounts of light and heavy particulate organic matter; (vi) no changes in the ratio of alkyl-C/O-alkyl-C over time; and (vii) light POM-C with a high C/N ratio. All of these factors together do not support the hypothesis that switchgrass favors the formation of stable macroaggregates and the sequestration of carbon in the short term. The perennial nature of switchgrass suggests that this native grass could have the potential to stabilize newly unstable small and large macroaggregates if the binding agents related with the inputs of organic matter increase as a consequence of an increase in the rate of residue decomposition over time. However, several researchers have reported that switchgrass can have positive effects in the short-term restoration of riparian zones because the living root systems of switchgrass remain in the soil to support regeneration of aboveground plant parts, and thus represent a continuous pool of stored carbon [13,29]. Also, the extensive root system of switchgrass is particularly effective in reducing nitrate-nitrogen loss in soil; the amount removed by switchgrass from the soil profile below 120 cm was 20 kg ha⁻¹ per year [31]. Nitrogen immobilization has also been measured during winter months [30]. Other important benefits of switchgrass are its effectiveness in reducing the transport of sediment. Lee et al. [32] reported that a 7.1-m wide switchgrass buffer alone was able to remove 95% of the sediment and 80% of sediment bound nutrients from adjacent row crop field runoff. In contrast, under the cropped system, every year soil perturbations inhibit the formation of new stable large and small macroaggregates and promote the disruption of unstable large and small macroaggregates, exposing particulate organic matter that had been protected and resulting in a loss of soil organic matter that further destabilize the stable macroaggregates. These riparian zones play an important role in stabilizing stream banks, provide sediment storage, serve as nutrient sinks for the surrounding watershed, and improve the quality of water leaving the watershed.

An important limitation of our analysis must be noted. We sampled the soil to a depth of 15 cm, but it is commonly thought that many of the benefits of perennials (C4) occur deeper in the soil horizon. However, there are at least two known mechanisms that promote soil organic carbon (SOC) sequestration: (1) ecotype-specific microbial communities can direct rhizodeposit C flow and C accrual deep in the soil profile [33]; (2) the SOC is added to the soil mainly by the deposition and decay of plant material on the surface and by root growth and senescence below the surface [18]. We applied the second method to assess soil aggrading through soil aggregation and particulate organic matter by riparian switchgrass buffers.

4. Materials and Methods

4.1. Site Description

The study sites were located within the small area adjacent to Bear Creek (42°09' N, 93°30' O), Long Dick Branch (42°10' N, 93°34' O), and Keigley Branch (42°07' N, 93°34' O) watersheds in north-central Iowa, USA. One plot of approximately 20 m × 30 m was located within each watershed, on a non-buffered field. We sampled three plots for switchgrass in a restored buffer that was established in 1990 along Bear Creek in areas that had been intensively grazed or cropped with corn and soybeans. The 20-m wide multi-species riparian buffer consisted of five rows of streamside poplars (*Populus × euramericana* 'Eugenei'), a row of ninebark (*Physocarpus opulifolius* L.), a row of redosier

dogwood (*Cornus sericea* L.), and finally a 7.3-m wide strip of switchgrass (*Panicum virgatum* L.) next to the cropped field. The annual corn-soybean rotation cropped plots were located along all three creeks, and the switchgrass was located at a restored buffer. All of the plots were located on the alluvial floodplain where the dominant soil type is Coland, a fine-loamy, mixed, superactive, mesic Cumulic Endoaquoll [34].

4.2. Experimental Design and Soil Sampling

The experimental design was a randomized complete block with three blocks represented by plots along three different creeks. Surface soil was taken from 20 randomly located soil cores within each plot once a month from March to November (nine consecutive months) for two consecutive years. We sampled the soil to a depth of 15 cm with a 5.6-cm steel coring bit for half of the cores and a 2.5-cm coring bit for the remaining 10 cores. The 10 cores taken with the larger diameter corer were pooled to form one composite sample from each plot. This sample was used to quantify aggregate size distribution, the stability of macroaggregates, and aggregate-associated soil organic carbon. The 10 cores taken with the smaller diameter bit were also pooled to form one composite sample and used to quantify carbon associated with the soil organic matter fractions. Soil samples were kept cool during transport and were stored in a refrigerator at 4 °C before processing and analysis [35].

4.3. Determination of Aggregate Size Distribution, Stability of Macroaggregates, and Aggregate-Associated Soil Organic Carbon

Determination of aggregate size distribution and stability of macroaggregates involves sorting the aggregates by their size and the macroaggregates by their water stability (slaking resistance). The aggregate size distribution and stability of macroaggregates were determined following the protocol developed by Márquez et al. [36]. The relative amounts of aggregates in each size fraction were expressed as a proportion of soil after sand correction. The redistribution of “loose sand” produces dispersion of C in the microaggregate size fraction and the enrichment of clay and silt in macroaggregate size fraction. Soil aggregates from each size fraction were ground by pestle in a mortar and subsampled to determine total organic C with a Carlo-Erba NA 1500 CN analyzer (Haake Buchler Instruments, Paterson, NJ, USA).

4.4. Fractionation of Particulate Organic Matter

The light particulate organic matter (LPOM) and heavy particulate organic matter (HPOM) were separated following the experimental method reported by Márquez et al. [35] and Cambardella and Elliot [37]. The field-moist soil was passed through a 2-mm diameter sieve; the larger pieces of stubble and roots were removed by hand, and the soil was air-dried. A 30-g subsample was dispersed with 100 mL of 5 g L⁻¹ sodium hexametaphosphate by shaking for 15 h on a reciprocal shaker. The dispersed soil samples were passed through a 53-μm sieve and rinsed several times with distilled water. The material retained on the 53-μm sieve was back-washed on to a 20-μm nylon filter, and a vacuum was used to remove excess water. The material was then rinsed into a 100-mL beaker with sodium polytungstate (1.85 g cm⁻³) to a volume of 50 mL. The samples were allowed to separate overnight at room temperature. The following day, the floating LPOM was aspirated, washed several times with water on a 20-μm nylon filter, and dried at 50 °C. The material that did not float at a density of 1.85 g cm⁻³ was back-washed onto a 20-μm nylon filter and washed several times with water. The material was rinsed into a 100-mL beaker with sodium polytungstate (2.22 g cm⁻³) and allowed to separate overnight at room temperature. The floating HPOM material was aspirated, washed, and dried at 50 °C. We determined the concentration of organic carbon associated with nondispersed soil (total soil organic carbon, TSOC) and the dispersed soil organic matter fractions: mineral (mineral associated carbon, MAC), LPOM (LPOM-C), and HPOM (HPOM-C) by dry combustion on a Carlo-Erba NA 1500 CN analyzer (Haake Buchler Instruments, Paterson, NJ, USA).

4.5. Chemical Composition of LPOM

We assessed the stability of organic carbon in ARC and SGB soil samples by evaluation of the potential for LPOM to decompose and by its chemical composition [22]. The chemical composition of LPOM samples in the ARC and the SGB filter soil were examined with a solid-state ^{13}C nuclear magnetic resonance (NMR) spectrometer. We did not treat LPOM samples with hydrofluoric acid because it does not have paramagnetic materials that could potentially interfere with this analysis.

The ^{13}C NMR spectra were obtained at 75.41 MHz with a Bruker MSL 300 NMR spectrometer (G.M.B.H. Bruker, Karlsruhe, Germany). Magic-angle spinning of the sample was maintained at about 4.0 kHz. The NMR spectra consisted of between 20,000 and 50,000 acquisitions with a contact time of 3 ms and a pulse repetition time of 4 ms. The NMR spectra were divided into four chemical shift regions according to the chemical types of carbon as follows: 0–46 ppm (alkyl), 46–110 ppm (O-alkyl), 110–164 ppm (aromatic), and 164–190 ppm (carbonyl) [22,38]. The relative intensities of the chemical shift regions were determined by integration of the NMR spectra over given chemical shift ranges after phase and baseline corrections [22,38]. Integration of the NMR spectra was done with OriginPro (Version 8, OriginLab Corporation, Northampton, MA, USA). Chemical shift assignments were externally referenced to liquid Me_4Si (tetramethylsilane, TMS).

4.6. Data Analysis

The statistical analysis was carried out with SPSS (Version 24, IBM, 2016, Armon, NY, USA). A one-way analysis of variance (ANOVA) was performed to determine whether the means of samples from ARC and SGB differed. We compared the effects of ARC and SGB treatments on MAC, LPOM-C, HPOM-C, the proportion of water-stable and water-unstable aggregates, and aggregate-associated organic carbon. An ANOVA result with a *p*-value less than the significance level of 0.05 leads us to conclude that the two means compared were statistically different. Before proceeding with the ANOVA, we tested the assumption of normality and homogeneity of variance of the residuals.

5. Conclusions

Seven years after the conversion of the ARC to SGB, there was no apparent difference between the formation of new stable large and small macroaggregates. Most of the new macroaggregates were unstable. We hypothesize that the lack of the relative instability of the new-formed macroaggregates may be attributed to the presence of: (i) coarse roots, (ii) higher C/N ratios in the light particulate organic matter, (iii) lower inputs particulate and heavy organic matter, and (iv) no changes in the alkyl-C/O-alkyl-C carbon ratio over time. There were no significant differences in total, light, and heavy particulate organic matter between the ARC and the 7-year-old SGB. It appears that several years of switchgrass will be required to realize a soil C sequestration benefit [13,18]. However, the potential effect of the SGB in slowing surface runoff and trapping sediment and in providing wildlife habitat provide positive benefits that have been reported in the same area early in the restoration process. Further study is warranted to test the long-term effects of SGB on carbon sequestration and aggregate stability.

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Conflicts of Interest: The authors declare no conflict of interest.

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