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Effects of traditional field retting of hemp on soil organic carbon and the soil microbial community

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Abstract

Retting is a process used with bast fiber crops such as industrial hemp (*Cannabis sativa* L.) to expedite the separation of fibers from the woody core of the stalk. Field retting involves partial decomposition of stalks on the soil surface and is facilitated by soil microorganisms. Effects of field retting on soil C have not been studied, and little information is available on how field retting affects the soil microbial community. We conducted an experiment to investigate effects of field retting on total organic soil C (TOC), permanganate oxidizable soil C (POXC), and relative abundance of microbial fatty acid methyl ester biomarkers (FAMEs). Four treatments were imposed: (a) no retting (NR), (b) low-density retting (LDR) (2,500 kg stalks ha^{-1}), (c) high-density retting (HDR) (5,000 kg stalks ha^{-1}), and (d) control (CON). The NR, LDR, and HDR treatments were conducted on plots where hemp was grown, whereas the CON treatment excluded production and retting. Plots under HDR treatment generally had greater TOC and POXC than plots where no retting occurred. Analysis of FAMEs indicated that soil microbial communities were grouped according to retting treatments. Greater fungal abundance and higher fungi to bacteria ratios were associated with soils where retting occurred. Compared with retting methods where harvested stalks are immediately removed from the field, field retting may mitigate soil C losses associated with harvest and may be a useful component of maintaining soil health when used in concert with management practices that build soil C.

1 | INTRODUCTION

Industrial hemp (*Cannabis sativa* L.) may play a role in meeting the increasing demand for biodegradable and sustainable fiber products (Shahzad, 2012). Legal changes in multiple countries over the last 25–30 yr have loosened restrictions on hemp production by differentiating it from *Cannabis* with higher levels of Δ9-tetrahydrocannabinol (THC), the primary intoxicating agent in *Cannabis*. Industrial hemp is generally legislated as *Cannabis* with less than 0.2–0.3% THC, depending on the jurisdiction. The changing laws have facilitated a global resurgence in consumer interest in hemp and hemp production (Mark & Snell, 2019). Among the modern fiber products being produced from hemp are construction and reinforcement materials, composite materials, textiles, and pulp and paper products (Shahzad, 2012). Given the increasing demand and modern uses, hemp fiber

Abbreviations: AMF, arbuscular mycorrhizal fungi; CON, control; FAME, fatty acid methyl ester biomarker; F/B ratio, fungal/bacterial fatty acid methyl esters; HDR, high-density retting; KSU, Kentucky State University; LDR, low-density retting; MRPP, multiresponse permutation procedure; NMS, nonmetric multidimensional scaling; NR, no retting; POXC, permanganate oxidizable soil carbon; SCC, St. Catharine College; SOM, soil organic matter; TOC, total organic soil carbon.

production is once again of interest, and new research and production efforts have grown accordingly in the United States and globally.

With the extended period of *Cannabis* prohibition in the 20th century, there are many unanswered agronomic questions about hemp cultivation in modern cropping systems. Where does this crop fit in modern crop rotations? What are best management practices for this crop in sustainable agroecosystems? What effects does hemp have on soil health? Building soil organic C is a key aspect of soil health in sustainable agroecosystems, fostering increased microbial activity, biodiversity, and associated nutrient availability (Weil & Magdoff, 2004). There is little information in the literature on the effects of modern hemp production on soil C and soil health. The only study on the subject that we could find was a review conducted by Adesina et al. (2020). They report on the soil health-building potential of hemp, but the literature they cite on this subject (e.g., Kraenzel et al., 1998) is somewhat dated preliminary work based largely on anecdotal evidence. Adesina et al. (2020) highlight the need for more agronomic research on hemp production and specifically call for research that promotes sustainable production.

Soil organic matter (SOM) is a key soil constituent that can be managed to maintain or promote soil health (Karlen et al., 1994; Weil & Magdoff, 2004). The amount of SOM is typically estimated through measurement of total soil organic C (TOC); however, TOC is often broken down into pools based on recalcitrance or lability of the C compounds in each pool (Parton et al., 1987; Weil et al., 2003). More labile pools of soil C tend to respond more quickly to management practices than TOC, and measurements of labile pools of soil C such as particulate organic matter (Marriot & Wander, 2006) and permanganate oxidizable C (POXC) (Culman et al., 2012; Lucas & Weil, 2012; Weil et al, 2003) have been used to monitor management-induced changes in soil C.

Plant residues contribute to building soil C (Franzluebbers, 2004; Lal, 1997) and also influence the soil microbial community (Lucas et al., 2014; Schutter et al., 2001). Microbial biodiversity and activity are directly related to soil C dynamics, and fungi in particular are thought to play a significant role in sequestering carbon and maintaining soil health (Beare et al., 1997; Six, et al., 2006). Microorganisms are involved in transformation of plant residue C into soil C pools (Moore-Kucera & Dick, 2008). Fungi have a critical role in that they simultaneously transform and translocate, via mycelia, residue C from litter on the soil surface into the upper layer of soil (Müller et al., 2017).

When bast fiber crops such as hemp, flax (*Linum usitatissimum* L.), and kenaf (*Hibiscus cannabinus* L.) are produced, the ultimate harvested product is the bast fiber portion of stalks. Bast fibers are phloem-associated structures, located just under the epidermis, that support the vascular tissue and strengthen the plant stalks (Crônier et al., 2005). Before these fibers can be collected and used, they need to be separated from the rest of the stalk. This is accomplished through a process known as retting, in which the pectin that binds the fibers to the core of the stalk is degraded. This degradation can be accomplished through various means, including chemical, enzymatic, water, and field retting (Donaghy et al., 1990; Tahir et al., 2011). For chemical and enzymatic retting (and sometimes water retting) stalks are removed from the field after harvest and submerged in tanks of chemicals, enzymes, and/or water. Chemical and enzymatic retting can be costly due to the cost of chemicals or enzymes (Tahir et al., 2011). Water retting involves anaerobic microbial degradation of pectin in stalks that are submerged in tanks, lakes, rivers, or other bodies of water. This process is associated with excessive odor and pollution from the anaerobic activity (Tahir et al., 2011).

Field retting (also known as dew-retting) is a traditional process by which hemp stalks are laid on the soil surface after harvest and allowed to partially decompose. This process is largely driven by soil microorganisms, particularly fungi that play an important role in breaking down pectin and other stalk components (Fernando et al., 2019). Kraenzel et al. (1998) note that "as much as two-thirds of the organic matter is returned to the soil if hemp is field retted"; however, as noted above, this is based on anecdotal evidence and we could find no attempts to examine the effects of retting on soil C content in the peer-reviewed literature. Assuming that field retting does leave significant amounts of organic matter in and on the soil, it seems likely that such an organic matter input could affect the soil microbial community.

Numerous studies have examined the microbial community and function within the plant stalks during the retting of bast fiber crops. Ribeiro et al. (2015), using a real-time polymerase chain reaction approach, observed that harvest date, retting duration, and soil type affected the proportional distributions of bacterial and fungal species within field retted hemp stalks. Chabbert et al. (2020), using a

Core Ideas

- There is a need for contemporary agronomic information on hemp production.
- Field retting of hemp promotes a soil microbial community with greater fungal abundance.
- Field retting of hemp may mitigate harvest associated soil C losses.
- Field retting may help maintain soil C levels when used with soil C building practices.
- Field retting may be a more sustainable practice for separating hemp fibers from stalks.

metagenomic approach, showed that within the first weeks of retting, microbial colonization of flax coincided with enzyme activity and changes in fiber properties within the stalks. In contrast, Law et al. (2020) using 16S ribosomal RNA gene amplicon sequencing found that abundance of *Chryseobacterium* increased over time, but community structure remained relatively consistent. They suggested that the bacterial community involved in retting is inherent in the stalks and little affected by soil. The importance of fungal activity in breaking down stalk materials (Fernando et al., 2019) and of environmental factors such as temperature and humidity (Bleuze et al., 2018; Réquilé et al., 2021) have been described. Other studies established that adding pectinase hydrolyzing bacteria or enzymes enhanced retting efficiency and fiber quality (Foulk et al., 2008; Moawad et al., 2019).

Although significant work has been done to characterize microbes within bast fiber crop materials during the retting process, little is known about effects of the field retting process on the soil microbial community. Given that plant residues cause shifts in soil microbial community structure (Lucas et al., 2014; Schutter et al., 2001), field retting of fiber crops might have an effect on the inherent soil microbial community. Using density gradient gel electrophoresis of DNA extracted from soil, Castaldini et al. (2001) detected a rapid change in the soil microbial community after applying a slurry of water collected from the water retting of hemp. The microbial community reverted to the soil's native composition within 2 mo. However, the microbial community in a slurry containing water-retting by-products is likely dominated by anaerobic bacteria (Djemiel et al., 2020) and is not representative of a community carrying out the aerobic decomposition of bast fiber crops during field retting. The differences in the soil microbial community with field retting have only been investigated in flax by Djemiel et al. (2017). In this groundbreaking study, high-throughput DNA sequencing was used to determine that microbial community diversity in the soil was greater than diversity observed in the plant, and the soil community structure varied at different sampling points in the retting process. Rotating stalks also induced changes in soil bacterial (but not fungal) community structure, whereas abundances remained constant. However, within the plant, structure of both bacterial and fungal communities showed changes, and abundance of Bacteroidetes increased while Proteobacteria decreased at later stages of the retting process. Enzymatic functions of the community and effect on fiber quality were the focus of Djemiel et al. (2017), and discussion of the implications of changes in the soil microbial community was limited. Given the renewed interest in hemp production, more information is needed on the specific effects of field retting on the soil microbial community. This is particularly important from an agronomic (or sustainability) perspective in that certain microbial groups (i.e., fungi favor soil C sequestration) (Beare et al., 1997; Six, et al., 2006). This information would ultimately facilitate agroecosystem management decisions for producers and others interested in systems approaches for maintaining or building soil health.

The objectives of this research were to investigate the effects of field retting of hemp on soil C dynamics and the soil microbial community. To achieve these objectives, an experiment was conducted in which different densities of hemp were distributed on soil after harvest. Soil C and soil microbial community measurements were conducted on soil samples collected immediately after the retting was completed. We examined retting effects on TOC, POXC, and microbial fatty acid methyl ester biomarkers (FAMEs). We hypothesized that soils where field retting was conducted would have greater soil C levels and a different microbial community structure compared with soils where retting did not occur.

2 | MATERIALS AND METHODS

2.1 | Establishment of the experiment

The experiment was conducted using a randomized complete block design within each of two sites over three years. General soil characteristics for each site are provided in Table 1. In 2015, the experiment was established in three blocks at St. Catharine College (SCC) in Washington County, Kentucky, in a Shelbyville silt loam soil (fine-silty, mixed, active, mesic Mollic Hapludalfs). In 2016, St. Catharine College ceased operations due to financial concerns, and the experiment was reestablished at the Kentucky State University Harold R. Benson Research and Demonstration Farm (KSU) in an Elk silt loam (fine-silty, mixed, active, mesic Ultic Hapludalfs). Four blocks were used at the KSU site. Blocks measured 9.1 m by 36.6 m and were subdivided into eight plots measuring 4.6 m by 9.1 m. Each block randomly received one of four treatments: hemp production with no retting (NR), hemp production with low-density retting (LDR), hemp production with high-density retting (HDR), or a control treatment where no hemp was produced or retted (CON). The SCC site was in pasture for over 40 yr prior to hemp production. The KSU site was used for production of certified organic grain and oilseed crops for 7 yr prior to this study. The KSU site is certified organic for crop production and, while not certified, the SCC site was managed in accordance with organic regulations.

2.2 | Crop production

Crop planting and harvesting dates are presented in Table 2. Crop management was handled in accordance with National Organic Program standards. The planting areas at both sites

TABLE 1 General soil properties for the fields used in this research at St. Catharine College (SCC) in 2015 and Kentucky State University (KSU) in 2016 and 2017

Site	Soil series and texture	Sand	Silt	Clay	pH	P	K	Mg	TOC
		%				mg kg^{-1}			g kg^{-1}
SCC	Shelbyville silt loam	8.8	68.1	23.1	6.2	17.9	76.4	190	13.2
KSU	Elk silt loam	14.3	69.0	16.7	5.5	274	202	157	17.7

Note. Soil properties were determined in the A horizon (depth range: 0–15 cm) before the establishment of the experiment.

TABLE 2 Hemp planting, harvest, and retting information at the two sites, St. Catharine College (SCC) and Kentucky State University (KSU), used in this study

Site	Year	Planting date	Harvest date	Harvested dry matter	Retting dates	Days retted	Soil sample date
				kg ha^{-1}			
SCC	2015	15 June	16 Oct.	3,842	19 Oct.–10 Nov.	22	11 Nov.
KSU	2016	9 June	11 Oct.	4,147	12 Oct.–10 Nov.	30	11 Nov.
KSU	2017	7 June	2 Oct.	4,014	3 Oct.–27 Oct.	25	27 Oct.

were prepped with a shallow roto-tilling prior to planting. Because the SCC site was coming out of pasture, that site was tilled with a moldboard plow prior to roto-tilling. We used an established European fiber cultivar (Futura 75), which was seeded at a rate of approximately 67 kg ha^{-1} and planted to a depth of 0.64 cm with a row spacing of approximately 18 cm. No supplemental fertilization, pest control, or irrigation was used at either site. Weed control, when necessary, was carried out during the first 4–5 wk postgermination using a stirrup hoe with a 12.7-cm blade. The CON plots were treated as unplanted fallow during the growing season. During the growing season, weeds were controlled in CON plots through periodic use of stirrup hoes. Plants were grown through the entire vegetative phase of their lifecycle and harvested in October of each year of the study, after approximately 7–8 wk of the reproductive phase. A sickle-bar attachment on a walk-behind tractor was used to cut hemp stalks at approximately 2.5 cm above the soil surface. To obtain stalk biomass yield estimates, all hemp was collected from a 3-m by 3.6-m area within the central portion of each plot where hemp was produced. This was weighed fresh to the nearest kilogram, then 15 stalks were randomly removed, weighed to the nearest 0.1 g, oven dried (65 ˚C for 72 h), and reweighed to estimate moisture content. The remainder of the plot was then harvested. All stalks, except those previously removed for moisture analysis, were collected by hand and redistributed on plots for retting as described below. Using the same production methods, additional hemp was produced beyond that in the research plots to ensure enough material was available for the retting treatments. A rye (*Secale cereale* L.)–vetch (*Vicia villosa* Roth) cover crop was planted in all plots each fall within 24–72 h after retting treatments, and subsequent soil sampling (see below) was completed.

2.3 | Retting process

As described above, there were four treatments in this study. No hemp was retted on plots that received the CON and NR treatments. Plots in the LDR treatment received approximately 2,500 kg ha^{-1} of hemp stalks, whereas plots in the HDR treatment received approximately 5,000 kg ha^{-1}. These rates were based on stalk biomass yields of the Futura 75 cultivar observed by Dr. David Williams in the University of Kentucky's 2014 production season (David Williams, personal communication, 2014). The University of Kentucky observations indicated that 5,000 kg ha^{-1} was a high yield rate in 2014. Since 2014 was the first year of hemp production in Kentucky after passage of the U.S. Agricultural Act of 2014, other Kentucky-specific data were not readily available when our study was initiated in 2015. Literature examined at the inception of the study indicated that 5,000 kg ha^{-1} of dry matter is a realistic (although possibly underperforming) yield for fiber hemp. This rate falls within the stalk biomass yield range of 4,000 to 12,000 kg ha^{-1} (depending on seasonal conditions and cultivar) reported in Oliver and Joynt (1999) for Canadian crops. Our HDR rate is also within the range of historic United States stalk biomass yields of 4,480 to 27,000 kg ha^{-1} (depending on seasonal conditions and cultivar) reported in Ehrensing (1998).

To accomplish the retting process, stalks were applied to the soil surface in October, within 1–3 days after harvest (Table 2). Stalks were applied to HDR and LDR plots at the rates described above. Stalks were evenly distributed across the plot. Retting was carried out for a duration between 22 and 30 d (Table 2), depending on seasonal moisture conditions (Supplemental Figure S1), with dryer conditions necessitating more time. Stalks were flipped approximately halfway

through the retting period to ensure even retting. After the retting period, all stalks were removed from plots and soils were sampled as described below.

2.4 | Soil sampling, processing, and analysis

To get a basic characterization of the fields (Table 1), initial soil sampling at both sites was carried out prior to experimental establishment (27 May 2015 at SCC; 31 May 2016 at KSU) by collecting soil cores to a depth of 30 cm from random locations within the fields. Initial parameters measured included particle size analysis, pH, P, K, Mg, and TOC. Methods used to determine these parameters are described below. After retted stalks were removed from the field, soil samples were collected within each treated plot at random locations to a depth of 10 cm. Post-retting soil sampling dates are given in Table 2. Fresh soil samples were split after sampling. Approximately 150 g of fresh soil was removed from each sample for analysis of soil microbial FAMEs and stored at −20 ˚C to minimize microbial activity after sampling. The remainder of each sample was stored at 4 ˚C until processing, which occurred within 15 d.

Prior to analysis, the soil samples stored at 4 ˚C were processed by passing them through a 4-mm sieve to remove coarse fragments and through a 2-mm sieve for homogenization. Soils were then air dried for 48 h. Basic soil properties (Table 1) were characterized by the Division of Regulatory Services at the University of Kentucky using methods described in Jones (1999), unless otherwise noted. Briefly, a calibrated pH meter and electrode was used to determine pH in a 1:1 soil/water paste. Mehlich III extraction and quantification via inductively coupled plasma spectrophotometry was used to characterize P, K, and other elements. Particle size analysis was performed using the micropipette method of Miller and Miller (1987). Total organic C was quantified using a LECO dry combustion instrument (Nelson & Sommers, 1982). Permanganate oxidizable C was measured using the method of Weil et al. (2003), modified, as described in Lucas and Weil (2012), to use 2.5 g of soil instead of 5 g.

Ester-linked FAME biomarkers were analyzed in the soil samples from the 2015 and 2016 seasons that were stored at −20 ˚C. We were unable to analyze the 2017 samples for FAMEs because a freezer malfunction and thaw caused the subsamples set aside for this analysis to become waterlogged. Extraction of FAME biomarkers, and assignment of nomenclature for these biomarkers, was conducted as described in and Schutter and Dick (2000). The FAME biomarkers were assigned to different microbial groups as described in the available literature. Briefly, assignments were as follows: fungi (linolenic acid, 18:2ω6c; Frostegård & Bååth, 1996; Zelles, 1999), bacteria (sum of i15:0, a15:0, 15:0, i16:0, 16:1ω7t, i17:0, a17:0, 17:0, cyl7:0, 18:1ω7c, and cyl9:0; Frostegård & Bååth, 1996), Gram-negative bacteria (sum of cy17:0 and 18:1ω7c; Allison et al., 2005; Frostegård et al., 1993), Gram-positive bacteria (sum of i15:0 and i16:0) (Allison et al., 2005; Frostegård et al., 1993), actinomycetes (sum of 10Me16:0, 10Me17:0, and 10Me18:0) (Zelles, 1999), and arbuscular mycorrhizal fungi (AMF) (16:1ω5c) (Olsson, 1999). Analyses were based on relative abundances of FAMEs, which were determined by dividing the value for each microbial group by the total FAMEs within a sample.

2.5 | Meteorological data

All meteorological precipitation and temperature data referenced in this work (Supplemental Figure S1) were collected from online sources. Monthly temperature and precipitation data were gathered via the Kentucky Mesonet system (Kentucky Mesonet, 2021). The data for SCC was gathered from the nearest Kentucky Mesonet collection location (37.63˚ lat., −85.37˚ long.) in Marion County, Kentucky, approximately 12 km southwest of the experiment site. The data for KSU were gathered from the Franklin County Kentucky Mesonet Station, which is located at the research site on the Kentucky State University Harold R. Benson Research and Demonstration Farm. Annual average temperatures and precipitation were gathered from the U.S Climate Data system (U.S. Climate Data, 2020), using the closest Kentucky locations to the research sites. The Bardstown location was used for annual averages for SCC, whereas Frankfort was used for KSU. Bardstown is located approximately 25 km west of the SCC site. The KSU site is located in Frankfort.

2.6 | Statistical analysis

For statistical analyses within an individual site, treatment effects on soil carbon parameters and individual microbial groups were analyzed via ANOVA and post-hoc Fisher's LSD means separation test. To analyze effects across all sites and years, we used a linear mixed model approach where the retting treatment was a fixed effect and site nested within year was a random effect. Post-hoc Fisher's LSD means separation tests were used to evaluate significant differences between treatment means when tests for fixed effects in the linear mixed model indicated significant treatment effects. Relationships between C parameters and selected microbial biomarkers were examined using simple Pearson's correlations. SYSTAT version 13 (SYSTAT Software) was used to conduct these analyses.

A multiresponse permutation procedure (MRPP) was used to determine whether microbial community composition was significantly different between the four retting treatments. The MRPP analysis was conducted as described in McCune et al.

(2002). An MRPP analysis uses a nonparametric multivariate approach to test for differences between two or more groups based on a matrix of Sorensen distances. To determine interrelatedness between retting treatments, soil C parameters, and microbial FAME biomarkers, a nonmetric multidimensional scaling (NMS) analysis was conducted, as described in MuCune et al. (2002). Interrelationships were illustrated in joint-plots. These analyses were conducted with PC-Ord version 5.1 (MJM Software). Relationships between variables and NMS ordination axes were examined using Pearson's correlations via correlations operation in PC-Ord. Because PC-Ord does not provide probability values, we tested significance with SYSTAT version 13 using the variables and NMS axis scores.

3 | RESULTS AND DISCUSSION

3.1 | Hemp yields and environmental conditions during retting

Adequate stands of hemp were established in each year of the study. There were no significant differences between hemp stalk biomass yields between different retting treatments in any year of the study. Average dry biomass yields for each year are given in Table 2; harvested stalks were used for retting treatments as previously described. Monthly precipitation data for 2015, 2016, and 2017, and deviations from monthly averages, are presented in Supplemental Figure S1. Hemp was retted for a slightly longer period of 30 d in 2016 to account for the dry conditions seen that fall (Supplemental Figure S1). Temperatures during the retting periods averaged approximately 13.7, 16.3, and 15.9 ˚C in 2015, 2016, and 2017, respectively. In each year, for the time periods associated with retting, these temperatures were above the annual averages for the study sites. Brief periods of freezing temperatures were observed on three occasions during the retting periods: −1.2 ˚C on 18 Oct. 2015, −1.8 ˚C on 10 Nov. 2016, and −1.2 ˚C on 26 Oct. 2017. Aside from freeze–thaw effects on stalks and fiber, we do not anticipate that these freezes of less than 12 h had a meaningful effect on the overall retting process.

3.2 | Effects of field retting on soil C

When examined across all years and sites, both TOC and POXC were significantly greater in HDR compared with other treatments (Figure 1). When analyzed individually within sites, HDR generally had the highest levels of C parameters. Field retting of hemp resulted in significantly higher TOC in HDR compared with CON or NR at the SCC site in 2015 (Table 3). At KSU, HDR had significantly greater TOC than CON in 2016, and in 2017, HDR had significantly greater TOC than CON and LDR. Although there were no significant differences due to retting treatments observed with POXC at SCC in 2015, POXC was significantly greater in HDR compared with CON and NR at KSU in 2016 and 2017 (Table 3).

It is not surprising that the retting process would affect soil C levels. Others have found that crop residues from harvested crops can have an effect on soil C. Rasmussen et al. (1980) found that addition of 5 metric tons of mature wheat (*Triticum aestivum* L.) crop residues (assuming a wheat C content of 42%) was enough to maintain soil C levels in a wheat–fallow cropping system imposed on a heavily tilled Mollisol soil. Karlen et al. (1994) found that TOC was higher in plots where "normal" and "double normal" input rates of postharvest corn (*Zea mays* L.) residues were left on a no-till soil, compared with "removal" plots where all residues were removed. Although no information on exact residue input rates or C content were provided, after 10 yr, the "normal" residue input plots had 80% greater C and the "double normal" plots had 155% greater C than "removal" plots. Clapp et al. (2000) found a 14% increase in TOC, measured to a soil depth of 15 cm, after 13 yr of continuous no-till corn production when corn stover was returned to the field. They attributed the increase in TOC to the addition of 32–42 mg ha^{-1} of C input (depending on corn fertilization rate) derived from the corn residue over the years of the experiment. Sharma et al. (2019) observed that TOC and labile C increased when crop residues were retained on the soil in a no-till rice (*Oryza sativa* L.)–wheat cropping system. They used assumed values (based on their review of the available literature) for C deposition from stubble, roots, and rhizodeposition and found that the rice–wheat system contributed 32.5 Mg ha^{-1} of C over 6 yr. According to Sharma et al. (2019), 0.9 Mg ha^{-1} came from wheat stubble and 15.7 Mg ha^{-1} was derived from rice stubble and residues. Numerous other examples of crop residues maintaining or building soil C stocks are given in Lal (1997), Wilhelm et al. (2004), Franzluebbers (2004), and Govaerts et al. (2009).

Although this research is the first examination, based on literature review, of effects of field retting of a bast fiber crop on soil C, the study had some limitations. In the residue studies discussed above, residues remained in the field; however, field-retted hemp stalks eventually are removed from the field for fiber extraction. Quantification of C transfer from hemp stalks to soil would have been useful in the context of our study, but due to labor and resource limitations, we were unable to measure the amount of C present in retted stalks before and after the retting process. For similar reasons, we also did not attempt to quantify specific soil C input associated with retting of hemp stalks or inputs from naturally senesced floral and leaf residues that had fallen from the stalks to the soil surface. Future studies would benefit from including such information.

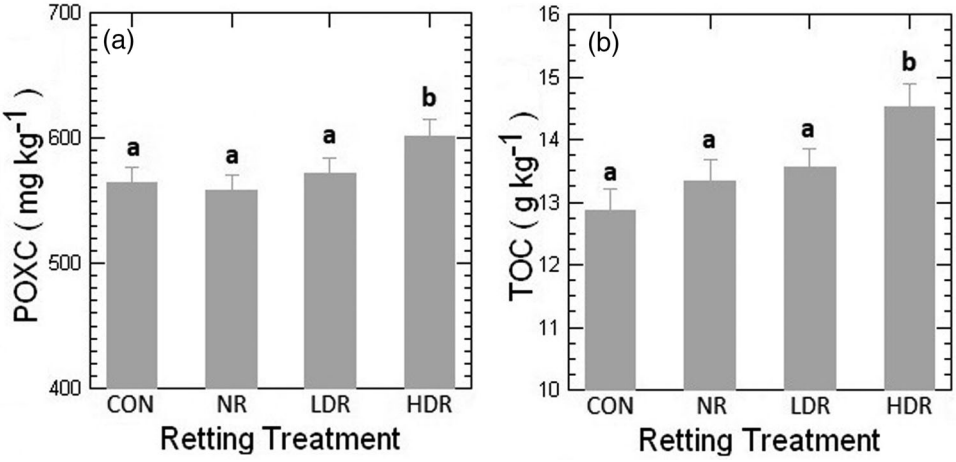

FIGURE 1 The effects of field retting on (a) permanganate oxidizable soil C (POXC) and (b) total soil organic C (TOC) across all sites and years in the experiment. Bars with different lowercase letters are significantly different at α = .05. Treatments included hemp production followed by field retting at a high stalk density (HDR); hemp production followed by field retting at a low stalk density (LDR); hemp production without subsequent retting (NR); and a control without production or retting (CON)

TABLE 3 Effects of hemp field retting treatments on permanganate oxidizable soil C (POXC) and total soil organic C (TOC) at St. Catharine College (SCC) in 2015 and Kentucky State University (KSU) in 2016 and 2017

			Treatment			
Variable	**Site**	**Year**	**Control**	**No retting**	**Low-density retting**	**High-density retting**
POXC, mg kg^{-1}	SCC	2015	605	588	608	652
	KSU	2016	581 a	581 a	590 ab	609 b
	KSU	2017	503 a	483 a	513 ab	541 b
TOC, g kg^{-1}	SCC	2015	12.0 a	12.1 a	12.9 ab	14.5 b
	KSU	2016	13.6 a	14.2 ab	14.2 ab	15.1 b
	KSU	2017	12.7 a	13.2 ab	12.7 a	13.7 b

Note. Means in the same row that have lowercase letters in common are not significant at α = .05. The absence of lowercase letters in a row indicates that no significant differences were observed.

Leaf senescence is a normal part of the maturation and flowering process in *Cannabis* and is often used as a visual indication that a plant is nearing the end of its life cycle (Frank & Rosenthal, 1990; Vogel, 2018). The C associated with these residues may have contributed to the observed increase in TOC, which was estimated on soil samples taken immediately after the retting process was completed. Soil TOC does not usually respond rapidly to management practices in the short term but rather takes several years to decades to change (Parton et al., 1987; Weil & Magdoff, 2004). On the other hand, POXC represents a relatively processed component of the labile soil C pool (Culman et al., 2012), and the potassium permanganate used for POXC analyses does not readily react with cellulose (Tirol-Padre & Ladha, 2004) such as that in fresh residues. The POXC results suggest that microbial activity on hemp residues may be promoting the soil C differences seen between retting treatments.

Future studies on effects of field retting on soil C would benefit from additional information on soil C dynamics. For instance, temporally distanced soil sampling events after the retting process is complete or quantification of C input from plant materials via either a mass balance approach carried out on the hemp stalks or via tracing C input using ^{13}C-labeled plant material (Herman et al., 2012; Moore-Kucera & Dick, 2008). Measurement of particulate organic matter C (Marriot & Wander, 2006), or light fraction C (Cookson et al., 2005), along with ^{13}C labeled hemp material could also add additional information to understanding the dynamics of soil C in response to field retting and retting associated litter deposition.

TABLE 4 Total fatty acid methyl ester biomarker (FAME) concentrations, fungi/bacteria ratio (F/B ratio), and relative abundances of FAMES associated with actinomycetes (Actino), arbuscular mycorrhizal fungi (AMF), bacteria, Gram-negative bacteria (Gram−), Gram-positive bacteria (Gram+), and fungi at St. Catharine College (SCC) in 2015 and Kentucky State University (KSU) in 2016

Site-year and treatment	Total FAME	Relative abundance						F/B ratio
		Actino	AMF	Bacteria	Gram−	Gram+	Fungi	
	μmol kg^{-1}	%						
SCC-2015								
Control	346.1	6.4 b	10.7	34.3	7.8	9.3	5.3	0.14
No retting	387.2	5.9 ab	10.9	33.1	7.2	9.1	5.8	0.18
Low density	399.7	5.4 a	10.5	33.1	8.4	8.7	6.6	0.20
High density	417.4	6.0 ab	10.6	33.7	7.9	9.1	6.1	0.18
KSU-2016								
Control	276.7	5.5	3.3 b	30.5	5.8 a	9.7 ab	8.7 ab	0.29 ab
No retting	290.5	5.7	3.1 ab	31.5	5.9 ab	10.0 b	7.9 a	0.25 a
Low density	289.2	5.5	3.2 b	30.9	6.3 bc	9.4 ab	9.1 b	0.30 b
High density	258.0	5.3	2.9 a	30.7	6.4 c	9.3 a	9.4 b	0.31 b

Note. Means in the same column that have lowercase letters in common are not significant at α = .05. The absence of lowercase letters in a column indicates that no significant differences were observed.

3.3 | Microbial community composition

Individual microbial FAMES are associated with different microbial groups, whereas the sum of microbial FAMES (total FAMES) can be used as a measure for microbial biomass (Allison et al., 2005; Frostegård & Bååth, 1996; Zelles, 1999). Analysis of variance conducted across both sites indicated that site was a significant driver of differences in all FAME parameters tested: total FAMES, AMF, fungi, bacteria, Gram-negative bacteria, and Gram-positive bacteria. ($p \leq .001$ for all tests). Significant treatment effects were also seen in the analysis across all sites. Fungal FAME relative abundance was significantly greater ($p < .04$) in HDR and LDR compared with CON and NR, whereas Gram-negative bacterial FAME relative abundance was significantly greater ($p = .014$) in LDR compared with NR. Significant site by treatment interaction was observed in the relative abundance of actinomycete FAMEs ($p = .008$) and in the ratio of fungal FAMEs to bacterial FAMEs (F/B ratio) ($p = 0.036$) indicating that treatment effects may have been behaving differently at each site with respect to these FAME parameters and thus precluding evaluation of treatment effects. These interactions are difficult to interpret. Examination of plots of least squares means plots for each site indicated that with actinomycete FAME relative abundance means for NR, LDR, and HDR were behaving differently relative to CON at each site. With the F/B ratio, examination of the least squares means plots indicated that LDR and NR seemed to be behaving differently between sites.

Examination of microbial FAME relative abundances within sites indicated few significant differences due to retting treatment effects at SCC (Table 4). The only significant difference observed was in actinomycete FAMEs, where CON had significantly greater levels than LDR. At the KSU site, more significant treatment effects were observed in 2016 (Table 4). There were significantly greater AMF FAMEs in CON compared with HDR. The HDR and LDR had significantly greater Gram-negative bacteria relative abundance than the CON, whereas NR had significantly greater Gram-positive bacteria relative abundance compared with HDR. At KSU in 2016, both HDR and LDR had significantly greater fungal FAME relative abundance and F/B ratio compared with NR.

The greater actinomycete relative abundance at SCC in CON compared with LDR is challenging to explain, given that other treatments where hemp was grown and/or retted showed no significant difference. The finding of greater AMF is interesting, given that CON plots were essentially kept fallow and AMF are known for associations with living plant roots (Schüβler et al., 2001). Although we did conduct weed control in CON plots, those efforts were not perfect, and conditions did allow for some weed growth, primarily Palmer amaranth (*Amaranthus palmeri* S. Watson), giant foxtail (*Setaria faberi* Herrm.), and Johnsongrass [*Sorghum halepense* (L.) Per.]. It is possible that that these weeds, having already had established populations in these plots, could have influenced the AMF population differently than the hemp, which had not been previously grown in the plots. Although AMF are generally thought to be non-host-specific, there is evidence that plants can influence AMF community structure (Horn et al., 2017).

The significant differences at KSU seem to indicate that retting treatments are changing the microbial community

composition toward a community that is proportionally higher in fungi. This finding contrasts somewhat with the findings of Djemiel et al. (2017), who saw consistent relative abundance of bacteria and fungi during the field retting process for flax stalks. Considering this contrast, it should be noted that Djemiel et al. (2017) was not comparing soil with retting to soil without retting, but rather observed temporal changes over the course of the retting process. Our findings agree with earlier works that showed that decomposition of plant residues can promote fungal abundance (Karlen et al., 1994; Lucas et al., 2014; Schutter & Dick, 2001). Fungi produce the extracellular cellulases, hemicellulases, lignin-peroxidases and manganese-peroxidases that facilitate decomposition of plant materials (Hammel & Cullen, 2008). Hammel and Cullen (2008) note that filamentous fungi in particular play a very important role as the primary decomposers of lignin. The primary components of hemp stalks are the hurd (comprising 60–80% of the stalk) and the bast fibers (making up 20–40% of the stalk) (Gümüşkaya et al., 2007). According to Gümüşkaya et al. (2007), hurd material is composed of approximately 40–48% cellulose, 18–24% hemicellulose, and 21–24% lignin, whereas bast fibers contain more cellulose (57–77%) but less hemicellulose (9–14%) and lignin (5–9%). Fungal activity is critical for decomposition of materials lying on the soil surface, as is the case with retting hemp stalks. Because these materials are not incorporated into the soil matrix, the fungal mycelia that bridge the interface between soil and stalks are an important aspect of the decomposition process (Müller et al., 2017). Müller et al. (2017) note that the mycelial connection at the soil–litter interface is critical in the translocation of C from litter material into the soil, a process which likely plays a role in the differences in soil C we observed in this experiment. Six et al. (2006) note that as soils become more fungal dominated, accumulation of stable SOM is favored. The greater abundance of Gram-negative bacteria in the LDH and HDH treatments may also be due to the ability of some Gram-negative bacteria to degrade lignin (Odier et al., 1981). Our findings are consistent with those of Herman et al. (2012), who observed that fungi and Gram-negative bacteria were primary consumers of ^{13}C-labeled litter within the first 21 d of deposition. Given our results and the findings of others with respect to the importance of fungi in the decomposition of plant residues in general (Lucas et al., 2014; Schutter & Dick, 2001) and in the retting process specifically (Fernando et al., 2019), tracking ^{13}C accumulation in fungal FAMEs and incorporation into fungal DNA (similar to Bernard et al. [2007]) could provide additional information on fungal C translocation processes and on specific fungi carrying out those processes. Additional research is also needed on where field retting might be most appropriate because the process is dependent on environmental factors such as suitable moisture for fungal proliferation (Sisti et al., 2018).

3.4 | Microbial community relationships with treatments and soil C

An MRPP analysis of the relative abundance of soil microbial FAMEs across both SCC in 2015 and KSU in 2016 revealed that, when both sites were analyzed together, the dominant factor determining grouping was site (test statistic $T = -35.45$; $p < .001$). An MRPP test of the retting treatment effect across both site-years was not statistically significant ($T = -0.11$; $p = .372$). In the test on the site effect, the groups were discretely separated based on site, likely masking other meaningful grouping comparisons across sites. We did not conduct NMS across both sites given these MRPP results.

When MRPP was conducted on soil microbial FAME composition structure at the individual site level, both SCC ($p = .0266$) in 2015 and KSU ($p = .0025$) in 2016 had significant grouping of microbial communities based on retting treatment (Table 5). When NMS was conducted on the microbial FAME relative abundances at SCC, a three-dimensional solution (final stress = 8.99) was determined to best explain the data. This determination was based on evaluation of a Scree plot of solution stress vs. number of dimensions. Cumulatively, the three-dimensional solution explained 92% of the variance in the data, with Axis 3 explaining 43%, Axis 2 explaining 21%, and Axis 1 explaining 28%. The best visual representation of the groupings at SCC was observed in an NMS joint plot of Axis 3 and Axis 1 (Figure 2a). The NMS of KSU microbial FAME relative abundances also indicated that a three-dimensional solution (final stress = 10.308) best explained the data, accounting for 90% of the variance. For the KSU data, Axis 3 explained 67% of the variance, whereas Axis 1 explained 15%, and Axis 2 explained 9%. The two dimensions that best visually represented the groupings at KSU were Axis 3 and Axis 2 (Figure 2b).

To aid in interpretation of the NMS results, we examined correlations of bioindicators and environmental variables with the various axes determined in the ordination (Table 6). Although patterns were, as expected, different between the two sites, one consistency between the sites was that fungal FAMEs and the F/B ratio were strongly and positively related to Axis 3. In examining the microbial communities associated with retting treatments, communities associated with LDR and HDR generally ordinate higher on Axis 3 (in the same direction as fungal FAMEs and F/B ratio; Figure 2). With the origin of the joint plot being the point at which the axes intersect, at SCC 83% of the LDR-associated microbial communities ordinate above Axis 2 (on the positive end of Axis 3), along with 50% of the HDR-associated communities. One HDR community lies directly on Axis 2. Meanwhile, 83% of the CON-associated and 67% of the NR-associated microbial communities ordinate below Axis 2 (on the negative end of Axis 3), with one NR community falling directly upon Axis 2.

TABLE 5 Results of multiresponse permutation procedure (MRPP) tests of soil microbial community differences between retting treatments based on fatty acid methyl ester biomarker (FAME) relative abundance at two sites: St. Catharine College (SCC) and Kentucky State University (KSU)

	Avg. within-group Sorensen distance					
Site-year	Control	No retting	Low-density retting	High-density retting	Test statistic (*T*)	*p* value
SCC-2015	0.44	0.50	0.57	0.32	−2.17	.0266
KSU-2016	0.42	0.39	0.50	0.42	−3.57	.0025

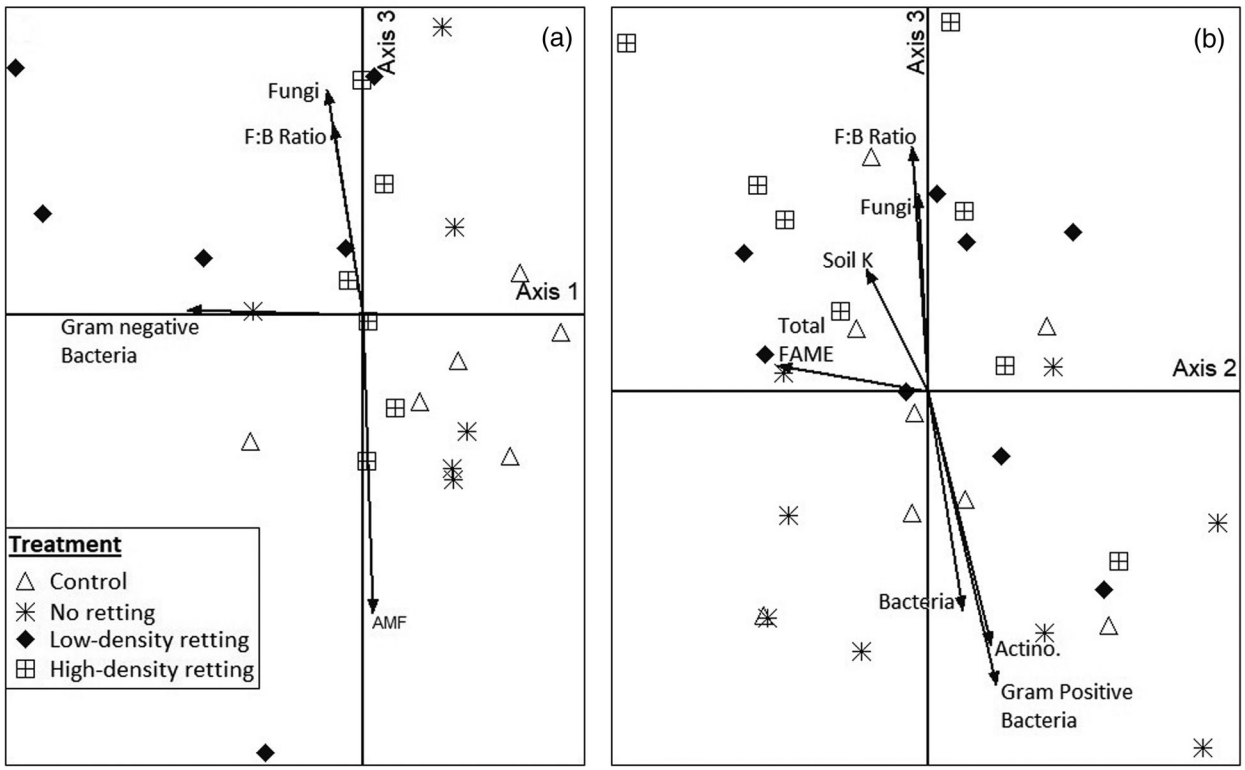

FIGURE 2 Nonmetric multidimensional scaling (NMS) ordination joint plots for two sites based on microbial fatty acid methyl ester biomarker (FAME) relative abundance. Joint plots show microbial community groupings based on hemp retting treatments for (a) St. Catharine College (SCC) and (b) Kentucky State University (KSU). Each geometric shape represents a microbial community profile associated with a given hemp retting treatment. The length and angle of vectors indicate the strength and direction of relationships between variables and ordination scores. Variables that showed relationships include actinomycete FAMES (Actino.), arbuscular mycorrhizal fungi FAMES (AMF), bacterial FAMES, fungal FAMES, fungi/bacteria ratio (F:B ratio), Gram-negative bacterial FAMES, Gram-positive bacterial FAMES, soil K, and total FAMES

Similarly, at KSU, 88% of the HDR-associated and 63% of the LDR-associated microbial communities ordinate on the positive end of Axis 3. One microbial community associated with LDR fell directly on Axis 2. In the CON-associated communities from KSU, 63% ordinate toward the negative end of Axis 3, whereas 75% of the NR-associated communities ordinate in this direction. These findings further support the idea that fungi are interacting with the hemp stalks and are important in the partial decomposition of these stalks. Other researchers have noted the importance of fungi in the retting process (Fernando et al., 2019).

Although both TOC and POXC were elevated in HDR at KSU and when analyzed across all sites (Table 3; Figure 1), there were no significant correlations observed between C parameters and fungal FAMEs or F/B ratio when sites were analyzed individually. However, TOC was positively related to both fungal FAMEs ($r = .445$; $p = .001$) and F/B ratio ($r = .434$; $p = .001$) when analysis was conducted across both sites. However, when relationships with POXC were analyzed across both sites, POXC had weak negative relationships with fungal FAMEs ($r = -.282$; $p = .035$) and F/B ratio ($r = -.301$; $p = .024$). One explanation for this might be related to the state of the hemp-derived organic matter in HDR

TABLE 6 Relationships between selected parameters and axes determined in the nonmetric multidimensional scaling (NMS) ordinations

Site-year and variable	NMS ordination axis		
	Axis 1	Axis 2	Axis 3
	correlation coefficient		
SCC-2015			
Actinomycetes	.444*	–.765***	–.424*
AMF	.165	.195	–.884***
Bacteria	–.014	–.851***	–.082
Fungi	–.313	.195	.810***
F/B ratio	–.285	.515**	.756***
Gram-negative bacteria	–.696***	–.351	.090
Gram-positive bacteria	.368	–.841***	–.320
pH	–.429*	–.295	–.002
Soil Mn	–.304	–.514**	–.211
Total FAMEs	–.244	.578**	.213
KSU-2016			
Actinomycetes	.040	.417*	–.837***
Bacteria	.165	.311	–.778***
Fungi	–.102	–.163	.740***
F/B ratio	–.122	–.211	.823***
Gram-positive bacteria	.096	.434*	–.900***
Soil K	.297	–.413*	.583***
Soil Mn	.229	–.435*	.278
Total FAMEs	–.129	–.644***	.270
Total organic C	.400*	–.268	.159

Note. Parameters that have at least one correlation coefficient ≥.40 (the lowest observed significant value at α = .05) are shown for the two sites used in the experiment. AMF, arbuscular mycorrhizal fungi; F/B ratio, fungal/bacteria ratio; FAME, fatty acid methyl ester biomarker.

*Significant at the .05 probability value.

**Significant at the .01 probability value.

***Significant at the .001 probability value.

and LDR. Observationally, hemp-related detritus remained on the plots after retted stalks were removed. This detritus, also known as light fraction organic matter, would combust and potentially show up in measurements of TOC. However, POXC represents a more processed pool of labile soil C and is not strongly correlated with light fraction material (Culman et al., 2012). Our observations (unpublished) in the laboratory corroborate the findings of Culman et al. (2012). We have observed (unpublished data) that when comparing soils heavily amended (0.01 g of vetch derived C g^{-1} soil) with fragments (<2 mm) of hairy vetch (*Vicia villosa* Roth.), amended and nonamended soils do not show a significant difference if tested side by side on the day the amendment is added; rather, some microbial processing must occur before POXC will show a difference. As fungi seem to be most important in the early stages of the retting process (Fernando et al., 2019), a different relationship between fungi and POXC might be seen if soil samples were taken at earlier stages of the retting process compared with what we observed when samples were collected within 24 h after field retting ended. More research needs to be conducted to fully understand the relationship between microbial processes and C translocation and transformation processes involved in field retting of hemp.

3.5 | Implications

Soil C is a key component of soil health in agroecosystems (Weil & Magdoff, 2004). Interest in maintaining or building soil C stocks has also been increasingly viewed as an important aspect in mitigation of global climate change (Lal, 1997). It has long been known that leaving residues on the soil influences soil C stocks (Karlen et al., 1994; Lal, 1997) and that microorganisms such as fungi and bacteria play a major role in facilitating carbon flow from surface residues to the soil (Müller et al., 2017; Six et al., 2006). The results of this study indicate that the traditional practice of field retting of hemp may influence C stocks in soils where this practice is used, with higher stalk density on the soil surface having a potentially greater effect on soil C content. Our outcomes also pro-

vide additional information on the importance of fungi in processing crop residues and in the field retting process.

Field retting of hemp alone is not a soil C building process, particularly when it is part of a production operation that will see most of the crop material removed from the field. In fact, in our study, TOC at the KSU site may have declined from the initiation of the experiment to the end of the first field season and again between 2016 and 2017, and at the SCC site, TOC may have also declined from initiation of the experiment to the end of the first season (Tables 1 and 3). We could not test the significance of the difference from initiation to the end of season in 2015 or 2016 because, for both sites, the initial data shown in Table 1 represents a whole field average from pooled soil samples collected across the entire field as opposed to samples from individual plots. However, at the KSU site, the loss of 1.2 g kg^{-1} of TOC between 2016 and 2017 (field TOC average = 13.1 g kg^{-1}) was significant ($p < .001$). Prior to this study, the field at the KSU site was used as a demonstration field for organic grain and oilseed crop production. These crops were produced in a minimum till system, tilled in at the end of the season, and followed by a rye vetch cover crop that was also tilled each spring. Similarly, at the SCC site, the field was converted from long-term pasture to hemp production. Changing from a less disturbed system with consistent residue inputs to a production system with increased tillage and crop residue removal may have promoted oxidation of TOC. Franzluebbers (2004) described in depth how tillage, disturbance, and changes in residue management can contribute to a decline in TOC. Our results suggest that the field retting process may mitigate some of the C loss associated with eventual removal of retted crop material as compared with other retting methods such as chemical retting, enzymatic retting, and water retting that involve complete removal of stalks from the field immediately after harvest (Donaghy et al., 1990; Tahir et al., 2011).

Field retting may leave a larger portion of hemp-derived C in the soil compared with immediate crop removal. Several studies (Jami & Kumar, 2017; Jasinskas et al., 2020) have found that mature hemp dry matter consists of approximately 45% C. Our study yielded dry stalk biomass rates ranging from 3,842 to 4,149 kg ha^{-1} harvestable hemp, depending on the site and year. Assuming 45% C, our harvests would represent a loss of 1,780–1,875 kg C ha^{-1} from the agroecosystem if hemp stalks were removed directly from the field with no retting. On the other hand, our HDR rate was at the lower end of the yield ranges observed in Canada and historically in the United States (Ehrensing, 1998; Oliver & Joynt, 1999). The retting rates used in this study were based on hemp yields observed in our region in 2014, the first year that hemp could legally be grown in Kentucky. These lower yield rates were likely due to modern inexperience with the crop in the region. With this in mind, our findings with respect to potential retting effects on soil C should be viewed conservatively. Further study using higher rates of material on the soil surface with other potentially higher yielding cultivars are needed.

Our results suggest that field retting might be used in combination with other management practices to maintain or enhance overall soil C content, thereby promoting soil health. Researchers have noted the challenges in maintaining soil C when the majority of the plant material is removed. Crops grown as bioenergy feedstocks may provide insight into management practices that could be used in conjunction with field retting, to maintain or build soil C, in hemp fiber production operations. Blanco-Canqui (2013) concluded that biofuel crop harvests that involve taking most of the plant can reduce soil C, but those losses can be mitigated through management. The mitigation can come through management practices such as no-till with cover crops, crop rotations that include periods of perennial plant growth, and/or additions such as animal manure, compost, or biochar. Blanco-Canqui (2013) notes that management practices may not be enough to offset soil C losses if excessive amounts of plant material are frequently being removed. With this in mind, producers interested in maintaining soil health may need to think carefully about how much biofuel or bast fiber plant material is removed. There is evidence that selective harvests that take less plant material and leave more crop residue in the field may mitigate C losses associated with crop removal. Osborne et al. (2014) observed that SOM and particulate organic matter fractions decreased as increasing amounts of corn stover were removed for biofuel feedstock.

On the other hand, the contribution of below ground biomass for hemp is not well characterized. Amaducci et al. (2008) observed extensive fibrous roots in the top 10 cm of the root zone and 50% of total hemp root biomass in the top 50 cm. They also observed tap roots extending to depths as deep as 200 cm. Finnan and Styles (2013) note that quantifying soil C storage attributable to hemp root deposition is challenging because hemp root system structure can vary greatly due to environmental factors such as soil compaction, high precipitation, or management influences such as tillage. Understanding hemp root systems with respect to soil C accumulation and rhizodeposition is a subject that needs more research. The problems associated with monoculture or short rotation cropping systems, including significant losses of soil C, have been well described (Bennett et al., 2012), and hemp grown for fiber would seem to be best suited as a crop to be grown as part of a diversified longer-term rotation that could include a perennial pasture phase. The findings of Sulc and Franzluebbers (2014) suggest that producing hemp in an integrated crop–livestock production system could also help offset soil C losses associated with removal of hemp biomass.

4 | CONCLUSIONS

With renewed interest in hemp production, sound agronomic information and information on the effects of hemp production on soil C dynamics and soil health is needed. This research provides new information on soil C and microbial dynamics associated with field retting of industrial hemp. Our results indicate that field retting might mitigate soil C losses associated with removal of the crop at harvest. Our results also provided evidence that field retting promoted a microbial community with greater fungal abundance. When used in concert with other soil C promoting practices, field retting of bast fiber crops could be an additional component in production systems that prioritize soil health and C sequestration. Although our findings provide a previously missing piece of information on how field retting effects soil C, additional research is needed on SOM stabilization, fungal role in hemp-derived C dynamics, and other management strategies such as crop rotation practices. The results of this research could help researchers, producers, and Extension professionals in making decisions on whether hemp grown for fiber is suitable for agroecosystems where soil C maintenance or accumulation is a concern.

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AUTHOR CONTRIBUTIONS

Shawn T. Lucas: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing. Anthony F. Silvernail: Data curation; Investigation; Resources; Visualization; Writing – review & editing. Michael D. Lewis: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Resources; Visualization; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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