

Impact of High-Lignin Fermentation Byproduct on Soils with Contrasting Organic Carbon Content

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Agricultural biomass is a potential renewable biofuel that may partially replace nonrenewable fossil fuels. Corn stover is rich in cellulose and hemicellulose, both of which can be converted to sugars and fermented to ethanol. This fermentation process results in a high-lignin fermentation byproduct (HLFB) that could be converted to energy products or used as a soil amendment. We had two objectives: (i) to determine whether HLFB (0.1, 1.0, or 10 kg m⁻²) could improve soil properties in two soils with contrasting levels of soil organic carbon (SOC); and (ii) to assess the impact of HLFB on crop growth. These goals were addressed with separate experiments. In the soil experiment, two soils were amended with HLFB or ground corn (*Zea mays L.*) stover and then incubated in pots for 118 d. Flux of CO₂ was monitored and soil properties were measured after incubation. In the plant experiment, corn and soybean [*Glycine max (L.) Merr.*] were grown in pots, without amendment or amended with 1.0 kg m⁻² corn stover or 1.0 kg m⁻² HLFB. The soil experiment indicated that the addition of 10 kg m⁻² HLFB increased CO₂ emission, humic acid concentration, and water-stable aggregates, and decreased bulk density (D_b). No adverse impacts on crop growth were measured when HLFB was applied at a rate of 1.0 kg m⁻². Much of the HLFB may be used by the energy industry, but perhaps a percentage could be returned to the field to reduce the impact of corn stover removal on soil C.

Abbreviations: HLFB, high-lignin fermentation byproduct; SOC, soil organic carbon.

Due to the unstable social and political conditions in many of the major oil-producing regions of the world, it is imperative to reduce dependence on foreign oil. Corn stover and other high-cellulose crops represent a domestic, renewable source of near-neutral C emission biofuel (e.g., ethanol, syngas) and biomaterial (Perlack et al., 2005; Ragauskas et al., 2006). A near C-neutral biomaterial acts neither as a net source of CO₂ nor as a CO₂ sink because the fermentation process uses C recently fixed by the material rather than releasing fossil C. Both the U.S. Department of Energy and private enterprise are working to develop fermentation processes that produce ethanol from high-cellulose biomass such as corn stover (Dipardo, 2000; Hettenhaus et al., 2000; Perlack et al., 2005). Iogen Corporation, a private company in Canada, is operating a demonstration-scale plant, with a capacity to process 30 Mg of feedstock d⁻¹ and produce 2.5 million L of ethanol yr⁻¹ (Iogen Corporation, 2005). They envision commercial facilities that could produce 75 million L yr⁻¹ of cellulosic ethanol.

The use of biomass for energy may partially offset energy requirements currently fulfilled by fossil fuels (Farrell et al.,

2006; Kim and Dale, 2005; Paustian et al., 1998). Lal (2004), however, concluded that biofuel produced from crop residue could not produce sufficient energy to make a major difference in reducing fossil fuel consumption and that its removal may seriously jeopardize soil and environmental quality. The efficacy of using biomass for energy has been questioned by some (Pimentel and Patzek, 2005). In contrast, Sheehan et al. (2002) reported that production of ethanol from corn stover used less energy input than that required for production of gasoline from crude oil. The disparity among these reports is due in part to how energy consumption during production is allocated between ethanol and coproducts in the net energy calculations (Sheehan et al., 2002). Farrell et al. (2006) reported that when coproducts (e.g., animal feed) were included in energy calculations, the net energy balance resulted in a production of 4 to 9 MJ L⁻¹ grain ethanol. Farrell et al. (2006) also noted that ethanol produced from corn grain reduced petroleum use by 95% compared with production of gasoline from crude oil. Ethanol production uses coal and natural gas in place of petroleum, however, which limits the reduction of greenhouse gas emission. They went on to suggest that cellulosic ethanol offers a significant reduction in greenhouse gas emission compared with gasoline production.

Some estimates of the amount of corn stover or wheat straw that can be removed for bioenergy use include a provision for leaving a percentage of corn biomass on the field to limit soil erosion (Farrell et al., 2006; Nelson et al., 2004), but do not consider the C inputs required to maintain SOC. Soil organic C must be maintained to sustain soil productivity (Kim and Dale, 2005; Perlack et al., 2005). Johnson et al. (2006) estimated an annual input of 2.1 to 3.0 Mg C ha⁻¹ from stover was required to maintain SOC levels for continuous corn. Assuming a stover yield of

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10 Mg ha⁻¹ with 400 g C kg⁻¹, 2.5 to 4.75 Mg stover ha⁻¹ yr⁻¹ could be removed while maintaining SOC. The range in values is attributed in part to variations among tillage systems and climate. Additionally, other management practices such as winter cover crops (Kim and Dale, 2005) could reduce the environmental risks of soil erosion and loss of SOC associated with harvesting biomass.

Numerous substances (e.g., manure, compost, organic waste) have been used as soil amendments. The response of soil properties to amendments varies with the characteristics of the amendment (Tejada and Gonzalez, 2006) and the soil (Schlecht-Pietsch et al., 1994). Composted cotton (*Gossypium hirsutum* L.) gin waste increased soil structure, microbial biomass, and decreased D_b while sugar beet (*Beta vulgaris* L. subsp. *vulgaris*) vinasse (the final byproduct after production of crystalline sugar, pulp, molasses, and alcohol fermented from pulp) had the reverse effect on these soil characteristics (Tejada and Gonzalez, 2006). The negative effects of beet vinasse were attributed to its high concentration of Na⁺ and fulvic acid.

Corn stover is about 60% cellulose and hemicellulose (U.S. Department of Energy Biomass Program, 2002), most of which should be converted to ethanol during fermentation; thus, there would be considerably less HFLB biomass compared with the original stover biomass. The byproduct remaining after cellulosic ethanol production, however, has a high lignin concentration (D. Schell, National Renewable Energy Lab, personal communication, 2002). Organic amendments that are high in lignin are expected to be biochemically recalcitrant (Haider and Martin, 1981; Stott et al., 1983) and have the potential to be physically isolated (Christensen, 1996). Both mechanisms can contribute to sequestering C (Palumbo et al., 2004). Johnson et al. (2004) demonstrated that the addition of HFLB to soils can increase humic acid concentration and increase the percentage of water-stable aggregates. The HFLB-amended soil enhanced soluble C, microbial biomass C, and CO₂ emission compared with unamended soil. Thus, amending soil with HFLB may partially offset the potential negative impacts of removing the corn stover.

Currently, no information is available regarding the potential impacts of HFLB on plant growth. The U.S. Department of Energy's National Renewable Energy Laboratory analyzed HFLB to determine fermentation efficiency and energy value. The presence of salts, acids, bases, organic compounds, etc., that could inhibit plant growth or soil processes is unknown. A byproduct from a similar process, starch fermentation, is dry-distiller's grain, which frequently is used as cattle feed. Our null hypothesis was that HFLB would not have a direct impact on

plant growth. We still thought it was important to assess the impact of HFLB on crops, however, as an early step in assessing HFLB for potential environmental concerns. The objectives of this study were to: (i) determine whether HFLB (0.1, 1.0, or 10 kg m⁻²) could improve soil properties in two soils with contrasting levels of SOC; and (ii) assess the impact of HFLB on corn and soybean growth.

MATERIALS AND METHODS

Soil Experiment

A soil experiment was conducted to evaluate the effect of HFLB and corn stover on soil properties. Soils used in this study included a Svea loam (fine-loamy, mixed, superactive, frigid Pachic Hapludoll) and Langhei loam (fine-loamy, mixed, superactive, frigid Typic Eutrudept). Soils were collected from the tilled layer (0–15 cm) of a field in west-central Minnesota (45°N, 96°W); the field was characterized by a complex soil association with undulating landscape as described by Johnson et al. (2004). The two loam soils are found within the same catena and have similar pH, but differ in C and N content (Table 1). Soils were air dried and ground to pass through a 3-mm sieve.

Soil N and C concentrations were measured using a LECO CN-2000 (LECO Corp., St. Joseph, MI). Inorganic C was determined as described by Wagner et al. (1998). Ammonium- and NO₃⁻-N concentration were measured using an Alpchem autoanalyzer (OI Analytical, College Station, TX), and pH in H₂O and in CaCl₂ were measured using a 2:1 water or buffer to soil ratio (Thomas, 1996). Humic acid was extracted according to Stevenson (1994) as modified by Johnson et al. (2004). Briefly, the soil was treated with 0.05 M HCl (5 L kg⁻¹ dry soil) to remove carbonates. Humic and fulvic acids were extracted with 0.5 M NaOH under N₂, and humic acid was precipitated by adjusting to pH 1 with HCl. In calcareous soils such as the Langhei, it is very difficult to remove all carbonates (Johnson et al., 2004), thus some carbonates probably remained in the Langhei soil.

Corn stover was ground (4-mm sieve) to a particle size comparable with HFLB, thus minimizing any differences in decomposition due to particle size. The HFLB was acquired from experimental cellulosic corn stover fermentation conducted at the U.S. Department of Energy's National Renewable Energy Laboratory in Golden, CO. Cellulosic fermentation converts most of the cellulose and hemicellulose to ethanol; therefore, HFLB had a greater concentration of lignin and N, but a lower concentration of cellulose and hemicellulose compared with corn stover (Table 2). The HFLB also had lower C/N and lignin/N ratios than corn stover.

Table 2. Characteristics of corn stover (CS) and high-lignin fermentation byproduct (HFLB).

Parameter	CS†	HFLB‡
C, g kg ⁻¹	470	590
N, g kg ⁻¹	7.0	20
Lignin, g kg ⁻¹	190	590
Cellulose, g kg ⁻¹	360	110
Hemicellulose, g kg ⁻¹	230	50
C/N	67	30
Lignin/N	270	30

† Average corn stover values reported by U.S. Department of Energy Biomass Program (2002).

‡ Composition analysis provided by Dan Schell at NREL, Golden CO; this analysis reported 12.4% protein concentration. We estimated N percentage by assuming protein is 16% N.

Table 1. Initial characteristics of two soils (Svea and Langhei) used in the soil incubation study before adding amendment.

Parameter	Svea	Langhei
Total C, g kg ⁻¹	27.4	31.5
Inorganic C, g kg ⁻¹	7.0	22.7
Organic C, g kg ⁻¹	20.4	8.9
Humic acid, g kg ⁻¹	17.1	1.72
Total N, g kg ⁻¹	1.8	0.8
NH ₄ ⁺ -N, mg kg ⁻¹	5.5	7.2
NO ₃ ⁻ -N, mg kg ⁻¹	12.4	3.7
pH in water	7.9	8.0
pH in CaCl ₂	7.3	7.4

Soil (1.5 kg) was not amended (control), amended with corn stover (1.0 kg m⁻²), or amended with HLFB (0.1, 1.0, or 10 kg m⁻²). The highest HLFB rate (10 kg m⁻²) was chosen to determine if changes in soil chemical and physical properties could be detected in a soil with relatively high inherent SOC. The amendment was mixed with the soil initially by hand and then poured three times through a riffle-style sample splitter (Model EI23–3052, ELE International/SOILTEST, Loveland, CO), which is designed to divide dry material uniformly into equal portions, and the mixture was recombined. After thorough mixing, the mixture was packed ($D_b = 1.2 \text{ g cm}^{-3}$) into pots (polyvinyl chloride cylinders with sealed bottoms, 10-cm diam., and 20-cm height). There was a total of 360 pots. Forty pots were sampled 1 d after wetting and used to determine baseline soil properties (total C, total N, organic and inorganic C, humic acid, NH_4^+ , NO_3^- , pH, D_b , and water retention) before incubation. The remaining 320 pots were incubated for 118 d. There were two identical pots and four replications for each of the 40 combinations of soil, amendment, and incubation treatment. At the final sampling, one set of pots was used to determine the percentage of water-stable aggregates and the other set of pots was used for D_b determination, water-retention characterization, and chemical analysis.

Limited availability of HLFB precluded plot-scale experimentation. Therefore, various incubation conditions were included in the experimental design to mimic some aspects of a field environment. The pots were incubated as follows: (i) ambient temperature with a range of 13 to 39°C and average of 27.1°C (Fig. 1) and variable volumetric water content from 22.1 to 34.5%; (ii) ambient temperature and near-constant volumetric water content ($34.3 \pm 3.1\%$); (iii) constant temperature ($21 \pm 2.2^\circ\text{C}$) and variable volumetric water content; and (iv) constant temperature and near-constant volumetric water content. Temperature was monitored with StowAway-TidbiT (Onset Computer Corp., Bourne, MA) data loggers; one data logger was located in each incubator and two data loggers were located at pot level in the laboratory. Volumetric water content of 35% corresponded to a water-filled pore space of 60%, based on a D_b of 1.2 g cm^{-3} for both soils. Ambient temperature was the temperature inside a building without air conditioning. Soil water was monitored by measuring the mass of the soil pots at least once per week. Water was added to pots with the near-constant volumetric water content treatment when soil volumetric water content dropped to or below 32%, while water was added to pots with the variable volumetric water content treatment when volumetric water content dropped below 23%.

Bulk density was measured by hand inserting preweighed stainless steel rings (5.0-cm i.d. by 3.2 cm deep) into the pots before initial wetting of the soil. The rings were positioned with the top of the ring 1 cm below the soil surface. At the time of sampling (about 24 h after initial wetting and after incubating for 118 d), the rings were extracted manually from the pots and the excess soil was trimmed level with the ends of each ring. These soil samples were also used to compare water retention characteristics. After trimming, the rings were placed on a porous ceramic plate with an initial bubbling pressure of 10 kPa. The plate and soil within the rings were allowed to saturate overnight and were then sequentially equilibrated for about 24 h at each pressure (10, 30, 50, and 100 kPa). Ring plus soil weights were obtained after equilibrating at each pressure. Following the 100-kPa measurements, soil dry weights were obtained after drying at 105°C for 24 h.

Carbon dioxide flux was measured using a $\text{CO}_2/\text{H}_2\text{O}$ analyzer (Model LI-6262 LI-COR, Lincoln, NE), a small soil chamber (Model 6000–09), a flow control unit (Model LI-670), and a data logger (LI-

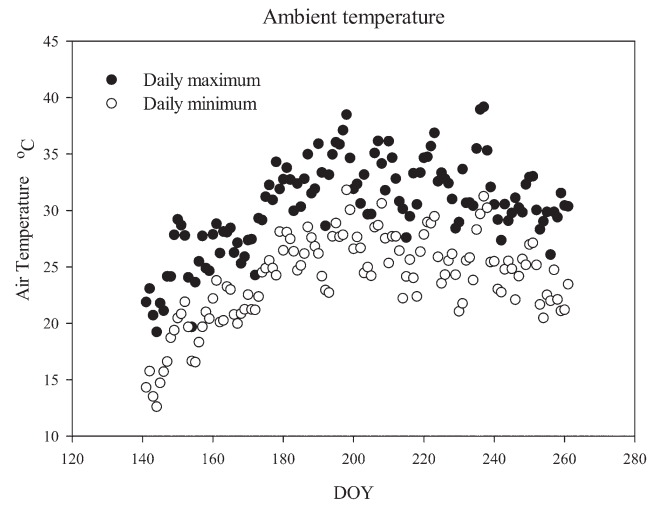


Fig. 1. Minimum and maximum daily air temperatures for soil pots incubated at ambient conditions. Temperatures ranged from 13 to 39°C between days of the year (DOY) 140 and 262.

6200) following standard operating recommendations (Dugas, 1993; LI-COR Biosciences, 1990). A thin, rubber gasket formed the seal between the soil chamber and the soil pot. The soil chamber attached to the data logger was flushed with ambient air and placed over the soil pot for data collection. After about 30 s, CO_2 flux was calculated from the rate of change of CO_2 concentration inside the chamber. Fluxes were measured 2, 3, 7, 8, 9, 13, 16, 17, 20, 22, 29, 38, 42, 51, 59, 64, 80, 86, 93, 101, and 112 d after starting the incubation. Sampling sequence was rotated to minimize potential diurnal flux bias. An average CO_2 flux rate ($\text{kg CO}_2 \text{ m}^{-2} \text{ h}^{-1}$) was calculated for each time interval by assuming linearity between consecutive sampling times. Cumulative CO_2 emission ($\text{kg CO}_2 \text{ m}^{-2}$) was calculated by first multiplying the average rate by the time interval and then summing over the course of the experiment.

Water stability of the 1- to 2-mm-diameter aggregates was determined in duplicate. Soil was air dried and then remoistened in a humidified wetting chamber to near field capacity. Soil was then wet sieved at 40 strokes min^{-1} for 5 min (Kemper and Rosenau, 1986).

Statistics

Soil pots were arranged in a $2 \times 2 \times 5$ factorial in a randomized complete block design within each temperature (Table 3). This design

Table 3. Analysis of variance model and error terms for testing the effect of temperature and independently testing the effect of soil water content, soil type, and amendment rate on soil properties within each temperature in a $2 \times 2 \times 5$ factorial experiment design.

Source of variation	Calculation of df	df
Between temperatures		
Replication (<i>R</i>)	$r - 1$	3
Temperature (<i>T</i>)	$t - 1$	1
Error	$(r - 1)(t - 1)$	3
Within temperature		
Replication	$r - 1$	3
Volumetric water content (<i>W</i>)	$w - 1$	1
Soil (<i>S</i>)	$s - 1$	1
Amendment (<i>A</i>)	$a - 1$	4
<i>W</i> × <i>S</i>	$(w - 1)(s - 1)$	1
<i>W</i> × <i>A</i>	$(w - 1)(a - 1)$	4
<i>S</i> × <i>A</i>	$(s - 1)(a - 1)$	4
<i>W</i> × <i>S</i> × <i>A</i>	$(w - 1)(s - 1)(a - 1)$	4
Error	$r - 1[(wsa) - 1]$	57

precludes comparison of temperature interactions with other factors (soil, water content, and amendment); however, the design does allow comparison among other possible interactions. The constant-temperature pots were incubated in four different incubators (treated as replicates). Pots were rotated within each incubator weekly to compensate for any internal temperature variations. The soil pots incubated at ambient temperatures were spatially randomized in four replications.

The effect of temperature was tested with the error term (temperature × replication; Table 3). Mean comparisons for water, soil, and amendment effects at each temperature were made with PROC GLM (SAS Institute, 2002). Linear regression analysis was used to investigate the relationships between amendment rate of HLFB and soil parameters (e.g., humic acid concentration and water-stable aggregates).

Plant Experiment

A plant experiment was conducted to evaluate the impact of HLFB on crop growth, which included three separate sets of plants. The growth of soybean was evaluated in a growth chamber, while the growth of corn was evaluated both in a growth chamber and under ambient conditions. Soybean and corn seed were sown in 19-L pots (27-cm diam. by 33-cm height) that allowed free drainage. Pots were amended with the following: (i) no amendment added (control); and (ii) amended with finely ground (4-mm sieve) corn stover (1.0 kg m⁻²) or HLFB (1.0 kg m⁻²). The corn stover rate of 1.0 kg m⁻² was equivalent to the amount of stover returned to the field after harvest of 10 Mg grain ha⁻¹. This assumes a harvest index of 0.50, which is slightly lower than that reported in Johnson et al. (2006). A grain yield of 10 Mg ha⁻¹ is representative of the 9 to 11 Mg ha⁻¹ yields reported for the U.S. Corn Belt in 2003 (National Agricultural Statistics Service, 2006). We chose to add HLFB at the same rate as corn stover to avoid confounding results due to rate differences. The amendments were mixed uniformly into the surface 15 cm of the pots to simulate incorporation by tillage in the field.

Corn

Corn was grown in a mix with equal parts by volume of Barnes loam (fine-loamy, mixed, superactive, frigid Calcic Hapludoll), peat moss, and sand. The peat moss and sand were used to improve drainage and rooting in the pots. Peat moss adds C, primarily as lignin and structural carbohydrates and other nutrients (Abad et al., 2002). Peat moss has the potential to confound the response to HLFB, which is also high in lignin (Table 2). Comparing treatments in the same potting mix, however, probably minimized this confounding effect.

Table 4. Analysis of variance table for comparing the effect of temperature, volumetric water content, soil type, and amendment rate on CO₂ released during a 112-d incubation period from soil cores amended with corn stover (CS) or high-lignin fermentation byproduct (HLFB).

Source of variation	df	P	P
Temperature (T)	1	****	
		Within temperature	
		Constant temperature	Ambient temperature
Volumetric water (W)	1	****	NS
Soil (S)	1	****	****
Amendment (A)	4	****	****
W × S	1	NS†	NS
W × A	4	NS	NS
S × A	4	*	*
W × S × A	4	NS	NS

**** Significant at the 0.0001 probability level.

* Significant at the 0.05 probability level.

† NS, not significant at $P \leq 0.05$.

Barnes loam was collected near Morris, MN, and had a history of continuous corn production for the past decade. Barnes loam was chosen for this experiment as it is a common, readily available soil in the area and has similar SOC levels and pH to a Svea loam (data not shown).

Initially, the pots were placed in a growth chamber programmed to 28/20°C and 16/8 h light/dark; the lighting was gradually increased or decreased during the initial and final light hours each day. Plants received a weekly 1-L application of 1 g L⁻¹ commercial fertilizer blend (20–19–18 N–P₂O₅–K₂O) as well as frequent irrigations to meet the evaporative demand. Fifteen days after planting, the corn was thinned to one plant per pot. Pots were then moved to a greenhouse with the same temperature and lighting period. Extended-leaf plant height and dry biomass was determined after 60 d.

We repeated the corn growth chamber experiment to verify results when growing the corn in ambient outdoor lighting and in a less consolidated potting mixture. Corn grown in the growth chamber exhibited symptoms related to consolidation of the soil mixture. Therefore, corn was grown in a 1:1 mixture of peat moss and sand to improve drainage. The pots were placed on wood pallets located on a sod surface out of doors. Plants were exposed to full sun for the duration of the experiment and watered every 2 to 3 d (unless it rained). Plants received an initial application (6 g per pot) of a slow-release commercial fertilizer (14–14–14 N–P₂O₅–K₂O) and then were fertilized weekly by applying 1 L of a 1 g L⁻¹ commercial fertilizer blend (20–19–18 N–P₂O₅–K₂O). Plant height (extended leaf) and dry biomass were measured at 66 d after planting. Corn was sown on day of the year (DOY) 162 and harvested on DOY 228.

Soybean

Plants were grown in a mix with equal parts by volume of Barnes loam, peat moss, and sand. Pots containing soybean plants were placed in a growth chamber programmed to 28/20°C and 16/8 h light/dark period. The lighting was gradually increased or decreased during the initial and final light hours each day. Soybean (three plants per pot) remained in the growth chamber for the entire experiment. Soybean received a weekly application of 1 L of a 1 g L⁻¹ commercial fertilizer blend (20–19–18 N–P₂O₅–K₂O) and was watered regularly to assure adequate soil water. Plant height and biomass were determined after 60 d.

Statistics

Separate ANOVA using PROC ANOVA (SAS Institute, 2002) was conducted for each plant–potting mix experiment to test the impact of amendment on plant growth. Each plant–potting mix experiment was arranged independently as a randomized complete block with five replications.

RESULTS AND DISCUSSION

Soil Experiment

Carbon Dioxide Flux

Carbon dioxide flux is indicative of soil respiration and associated decomposition. The pattern of cumulative CO₂ flux fit a double exponential function for all treatments (data not shown). Such a function describes decomposition both biologically and mathematically, partitioning the material into rapidly and slowly decomposing fractions (Wieder and Lang, 1982). This or similar kinetics models commonly are used to describe decomposition (Paul and Clark, 1996). Similar flux patterns were observed by Johnson et al. (2004). The CO₂ evolved from the soil pots differed ($P \leq 0.05$) between temperature treatments (Table 4). The soil incubated at nearly

Table 5. Carbon dioxide released over a 112-d incubation period from soil cores amended with corn stover (CS) or high lignin fermentation by-product (HLFB) as influenced by volumetric water content, soil type, and amendment rate.

Treatment			Cumulative CO ₂ release			
			Constant [†] temperature		Ambient temperature	
			kg CO ₂ m ⁻²			
Volumetric water ‡	Constant		1.54 a [§]		1.15 a	
	Variable		1.74 b		1.14 a	
Soil	Svea		1.89 a		1.28 a	
	Langhei		1.39 b		1.01 b	
Soil	Amendment	Rate [¶] kg m ⁻²	kg CO ₂ m ⁻²	% of Control	kg CO ₂ m ⁻²	% of Control
Svea	Control	0	1.25 cd	100	0.73 de	100
	HLFB	0.1	1.23 cd	98	0.71 e	97
	HLFB	1.0	1.51 cd	121	0.93 d	127
	HLFB	10	3.12 a	250	2.39 a	327
	CS	1.0	2.33 b	186	1.64 b	225
Langhei	Control	0	0.73 e	100	0.44 f	100
	HLFB	0.1	0.77 e	107	0.43 f	98
	HLFB	10	1.11 ed	152	0.62 ef	141
	HLFB	10	2.74 a	375	2.32 a	527
	CS	1.0	1.59 c	218	1.23 c	280

† Constant temperature (21 ± 2.2°C); ambient temperature (ranged 13 to 39°C).

‡ Constant water (34.2 ± 3.1 v v⁻¹); variable water (22.1 to 34.5 v v⁻¹).

§ Values within a temperature effect (in a column) followed by a different letter are significantly different at P ≤ 0.05.

¶ 0.1 g cm⁻² is equivalent to 10 Mg ha⁻¹.

constant temperature evolved 1.64 kg CO₂ m⁻², while soil incubated under ambient conditions evolved only 1.14 kg CO₂ m⁻². Although the ambient conditions had a higher average temperature, there were episodes of nonoptimal (too hot or too cold) temperatures (Fig. 1). The optimal temperature for decomposition is about 30°C (Paul and Clark, 1996).

Soils incubated at constant temperature under variable volumetric water content evolved 11% more CO₂ after 112 d than did soils incubated under near-constant soil volumetric water content (Tables 4 and 5). In contrast, soils incubated at ambient temperature evolved the same CO₂ when subjected to either variable or near-constant volumetric water content. Adding HLFB or corn stover increased CO₂ emission irrespective of soil type or water content treatment when averaged across soils (data not shown).

Of the potential interactions within each temperature treatment, only the soil × amendment interaction was significant (P ≤ 0.05; Table 4). Mean comparison in CO₂ emission from the soil and amendment treatments indicated significant differences between the control and addition of corn stover or 10 kg m⁻² HLFB (Table 5). The cumulative CO₂ emission in the control pots (Table 5) was consistent with the different SOC levels observed between soil types, in that more CO₂ evolved from the Svea than the Langhei soil (Table 1). Relative to the control, the cumulative CO₂ evolved from the Langhei soil was always greater than from the Svea soil. For example, the addition of 10 kg m⁻² HLFB increased CO₂ flux by 527% relative to the control for the Langhei soil, but only by 327% in the Svea soil when both soils were incubated at ambient temperatures (Table 5). This finding implies that Langhei soil is more responsive to the addition of HLFB.

Within the HLFB amendment treatments, the amount of CO₂ evolved was proportional to

the amount of HLFB added (Table 5) when both soils were subjected to constant- or variable-temperature regimes. Adding 1.0 kg m⁻² of stover resulted in 40 to 100% more CO₂ emission during the 112-d incubation period compared with amending with 1.0 kg m⁻² HLFB. The HLFB was expected to be more recalcitrant to microbial breakdown due to its large concentration of lignin, despite HLFB having lower C/N and lignin/N ratios than corn stover (Table 2). Decomposition is a function of substrate quality. Material with large C/N ratio, lignin/N ratio, or lignin concentration are more likely to decompose more slowly than comparable material (Berg and Matzner, 1997). In our study, differences in lignin concentration appeared to contribute to the slower decomposition of HLFB.

Bulk Density and Water Retention

Initial (after amending but before incubation) D_b differed between soils and among amendment treatments, but the interaction was not significant (Table 6). The target D_b when filling the pots with soil was 1.2 g cm⁻³, which was achieved

Table 6. Bulk density (D_b) and volumetric water content, equilibrated at retention pressures from 10 to 100 kPa, within 24 h of amending soil at different rates of corn stover (CS) or high-lignin fermentation byproduct (HLFB).

Treatment	Rate [†]	D _b	Volumetric water content			
			10 kPa	30 kPa	50 kPa	100 kPa
			m ³ m ⁻³			
Soil						
Svea		1.17 b [‡]	0.35 a	0.27 a	0.25 a	0.22 a
Langhei		1.24 a	0.33 b	0.27 a	0.25 a	0.22 a
Amendment						
Control	0	1.22 ab	0.33 b	0.25 b	0.24 b	0.21 c
HLFB	0.1	1.24 a	0.33 b	0.26 b	0.24 b	0.21 bc
HLFB	1.0	1.23 ab	0.33 b	0.27 b	0.24 b	0.22 b
HLFB	10	1.14 c	0.37 a	0.31 a	0.28 a	0.26 a
CS	1.0	1.20 b	0.33 b	0.27 b	0.24 b	0.21 b

† 1.0 kg m⁻² is equivalent to 10 Mg ha⁻¹.

‡ Values within a column followed by a different letter are significantly different at P ≤ 0.05.

for all treatments except the 10 kg m⁻² HLFB treatment. The initial water retention characteristics (Table 6) reflect the lower D_b of the 10 kg m⁻² HLFB treated soil. Indeed, the higher water-holding capacity of the 10 kg m⁻² HLFB treated soil across a range in water potential (10–100 kPa) is indicative of the higher porosity and lower D_b than the other treatments.

After incubating for 118 d, there was a small but significant ($P \leq 0.05$) difference in D_b associated with the temperature regime (Table 7). Within each temperature treatment, there were no significant ($P \leq 0.05$) interactions among the potential two- or three-way interactions (data not shown). Therefore, only the main effects of water, soil, and amendment are presented (Table 7). The variable water content treatment had a lower D_b than the constant water content treatment. The D_b for Svea loam was 1.18 ± 0.01 g cm⁻³ and for Langhei loam was 1.24 ± 0.01 g cm⁻³ (Table 7) when incubated at constant temperature. No differences in D_b were apparent between soils (1.18 g cm⁻³) when they were incubated at ambient temperatures. For both the variable- and constant-temperature treatments, the impact of 10 kg m⁻² HLFB on D_b and water retention persisted during the course of the incubation period (Table 7). For example, D_b of the 10 kg m⁻² HFLB treatment was significantly lower than

the other amendment treatments. In addition, soils amended with 10 kg m⁻² HFLB retained more water across a range in water potential (10–100 kPa) than soils either not amended or amended at other rates of HFLB (Table 7). Adding HFLB at very high rates potentially is akin to adding a low-density material such as peat to soil. Addition of low-density material to soil reduces consolidation and improves water-holding characteristics (Tables 6 and 7). We speculate that the reason the 10 kg m⁻² HFLB treatment resulted in significantly lower D_b (Table 6) than other amendment treatments even after 118 d of incubation (Table 7) was that sufficient amounts of this low-density material had not decomposed and thus it still impacted D_b . This observation is consistent with the CO₂ flux data, which suggests that about 5% of the 10 kg m⁻² HFLB decomposed during the incubation (Table 5). In contrast, as much as 30% of the corn stover decomposed in 118 d.

The impact of incubation condition (temperature or water content) on D_b was unexpected; however, more CO₂ evolved from soils incubated at constant temperature than from soils incubated at ambient temperatures (Table 5). Thus, soils incubated at constant temperature lost more organic material during this study. This reduction in organic material may have

resulted in an increase in D_b . The constant water content treatment, when incubated at constant temperature, also released more CO₂ than the variable water content treatment. Again, a reduction in organic material may have resulted in an increase in D_b for the constant water content treatment. Wetting and drying cycles associated with the variable water content treatment caused changes in soil structure (Dexter, 1991) that also probably contributed to the reduction in D_b relative to the soil maintained at near-constant water content.

Water retention characteristics reflect the ability of a soil to store and release water. Soil texture, clay mineralogy, and organic matter content impact soil water characteristics. The texture of the two soils is similar: Svea has 43, 38, and 19% sand, silt, and clay, respectively (Johnson et al., 2004). Soil incubated at ambient temperature consistently had less volumetric water at each retention pressure than soil incubated at a relatively constant temperature (Table 7). Within each temperature treatment, the potential interactions were not significant; therefore, only the main effects of water, soil, and amendment are reported (Table 7). For both temperature treatments, soils subject to the variable water content treatment retained less water at each retention pressure than soils subject to the constant water content treatment. We are unsure why the ambient temper-

Table 7. Bulk density and volumetric water content, equilibrated at retention pressures from 10 to 100 kPa, of soils after incubating for 118 d following the addition of corn stover (CS) or high-lignin fermentation byproduct (HLFB) as influenced by temperature, water content, soil type, and amendment rate.

Treatment	Rate†	D_b	Volumetric water content			
			10 kPa	30 kPa	50 kPa	100 kPa
	kg m ⁻²	g cm ⁻³	m ³ m ⁻³			
Temperature‡						
Constant		1.21 a§	0.31 a	0.27 a	0.25 a	0.23 a
Ambient		1.18 b	0.28 b	0.26 a	0.24 b	0.22 b
Constant temperature						
Volumetric water content¶						
Constant		1.25 a	0.33 a	0.30 a	0.28 a	0.25 a
Variable		1.16 b	0.29 b	0.25 b	0.23 b	0.21 b
Soil						
Svea		1.18 b	0.31 a	0.28 a	0.22 a	0.23 a
Langhei		1.24 a	0.30 b	0.27 b	0.20 b	0.23 a
Amendment						
Control	0	1.26 a	0.30 b	0.27 b	0.25 b	0.23 b
HLFB	0.1	1.25 a	0.31 b	0.27 b	0.25 b	0.23 b
HLFB	1.0	1.22 a	0.30 b	0.27 b	0.25 b	0.23 b
HLFB	10	1.01 b	0.32 a	0.29 a	0.27 a	0.25 a
CS	1.0	1.21 a	0.30 b	0.27 b	0.25 b	0.23 b
Ambient temperature						
Volumetric water content¶						
Constant		1.20 a	0.30 a	0.27 a	0.26 a	0.24 a
Variable		1.16 b	0.28 b	0.25 b	0.23 b	0.21 b
Soil						
Svea		1.18 a	0.30 a	0.28 a	0.25 a	0.23 a
Langhei		1.18 a	0.28 b	0.25 b	0.23 b	0.22 b
Amendment						
Control	0	1.20 a	0.29 bc	0.25 bc	0.24 bc	0.21 bc
HLFB	0.1	1.22 a	0.29 b	0.26 b	0.24 bc	0.22 bc
HLFB	1.0	1.19 a	0.29 b	0.26 b	0.24 bc	0.22 b
HLFB	10	1.11 b	0.32 a	0.28 a	0.27 a	0.25 a
CS	1.0	1.17 a	0.28 c	0.25 c	0.23 c	0.21 c

† 1.0 kg m⁻² is equivalent to 10 Mg ha⁻¹.

‡ Constant temperature (21 ± 2.2°C); ambient temperature (range 13–39°C).

§ Values within a column of a main effect within each temperature followed by a different letter are significantly different at $P \leq 0.05$.

¶ Constant water content (34.2 ± 3.1 v/v); variable water content (22.1–34.5 v/v).

ature and variable water content treatments retained less water at low pressures in this study. The D_b of these treatments was smaller than the D_b of the constant temperature and constant water content treatments; thus, porosity and the amount of water retained near saturation was expected to be greater for the ambient temperature and variable water content treatments. Other soil physical properties however, that affect water retention and were not measured in this study (e.g., pore size distribution) could have been influenced by the temperature and water content treatments. Svea loam with more SOC held more water than Langheii loam at low retention pressures irrespective of incubation temperature treatment. An extreme amount of HLFB (10 kg m^{-2}) was required to increase the amount of water retained at each retention pressure compared with the control or corn stover (Table 6). The addition of organic material such as corn stover or HLFB can impact water characteristics by altering porosity and by providing additional water binding sites. Other researchers (e.g., Barzegar et al., 2002) have observed a positive response in soil-water retention to the incorporation of wheat (*Triticum aestivum* L.) straw to a field soil in Iran. Water-holding capacity also tends to increase with increasing SOC (Hudson, 1994; Olness and Archer, 2005).

Humic Acid and Water-Stable Aggregates

There were no significant differences ($P \leq 0.05$) in humic acid concentration between incubation temperatures or soil volumetric water content treatments (data not shown). In addition, only the soil \times amendment interaction was significant ($P \leq 0.05$). This interaction was evident in different slopes and intercepts of the humic acid vs. HLFB relationship between the two soil types (Fig. 2). The Svea soil had inherently more humic acids than the Langheii soil (Table 1); this was in agreement with Johnson et al. (2004). Soil humic acid concentration, after the 118-d incubation, increased linearly as a function of the amount of HLFB added to both soils (Fig. 2). Previously, Johnson et al. (2004) demonstrated that about 30% of the HLFB could be recovered by our humic acid extraction method. Therefore, the increases in crude humic acid do not reflect necessarily humification of the HLFB to humic acid. Rather, it reflects the chemical similarity between crude humic acid and HLFB. In our previous study (Johnson et al., 2004), the increase in humic acid was only observed in a Langheii soil, presumably because the effect of HLFB in the Svea soil was not measurable against an inherently high humic acid content.

At the end of the experiment, there was a strong positive relationship between the concentration of humic acid and water-stable aggregates in both soils. By definition, water-stable aggregates cannot exceed 100% of total aggregates; therefore, we used a logarithmic function to describe the relationship between humic acid and water-stable aggregates (Fig. 3). Previously, we had observed a similar relationship for Langheii soil amended with HLFB (Johnson et al., 2004). In the current study, we also measured an increase in humic acid concentration in the Svea soil after adding a very large amount of HLFB, which was not observed with the lower amendment additions used in our previous study (Johnson et al., 2004).

Soil amended with corn stover had 93% water-stable aggregates, which was significantly greater than 88% for the control and 90% when amended with 0.1 kg m^{-2} of HLFB ($P \leq 0.05$). The HLFB (10 kg m^{-2}) amended soil had 94% water-stable aggregates, which was similar to amending with 0.1 kg m^{-2} of corn stover. Presumably, differences in the chem-

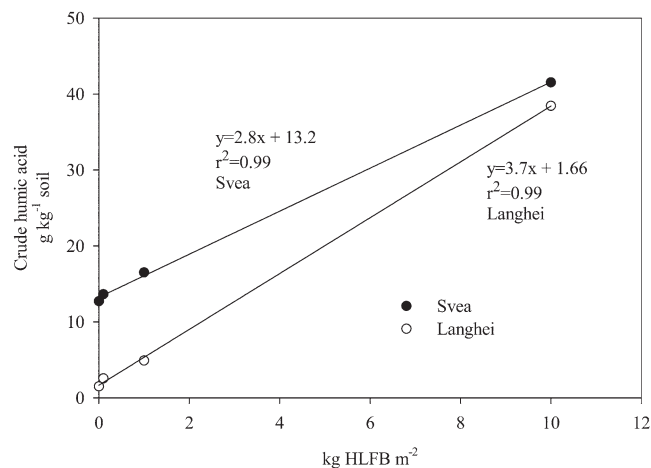


Fig. 2. Crude humic acid extracted after 118-d incubation of the two soils (Svea and Langheii) with high-lignin fermentation byproduct (HLFB).

ical composition (Table 2) and rate of decomposition (Table 5) between corn stover and HLFB affect the ability of these materials to alter the number of water-stable aggregates.

Tisdall and Oades (1982) demonstrated that water-stable aggregates tend to increase with organic matter concentration. Water stability of soil aggregates depends on organic materials such as polysaccharides, roots, fungal hyphae, and aromatic compounds (Tisdall and Oades, 1982). In addition, improvement of soil aggregate stability results from microbial utilization of carbohydrates and from plant phenolics, which are released during decomposition of structural components (e.g., lignin) (Martens, 2000). The apparent difference in the water-stable aggregates in soil amended with corn stover compared with that amended with HLFB may in part reflect differences in carbohydrate (cellulose and hemicellulose) and lignin concentrations (Table 2). Enzymatic fermentation during cellulosic ethanol production removed most of the carbohydrates; the absence of carbohydrates could directly or indirectly limit soil aggregation compared with corn stover via reduced microbial activity as indicated by decreased CO_2 flux (Table 5). Previously, Johnson et al. (2004)

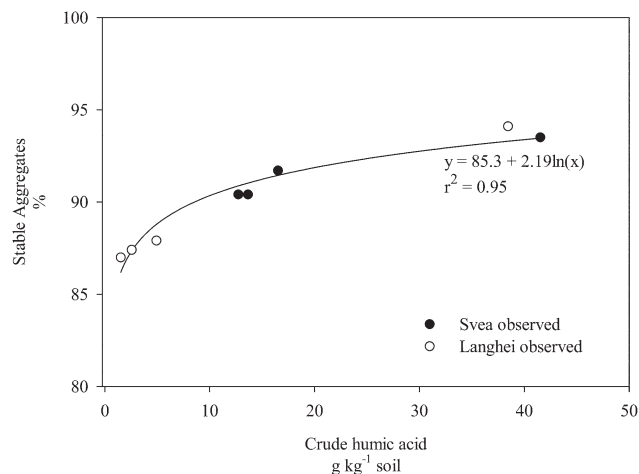


Fig. 3. Percentage of water-stable aggregates measured on air-dried and remoistened soil as a function of crude humic acid concentration in the two soils amended with high-lignin fermentation byproduct (HLFB) and incubated for 118 d. Regression equation calculated based on observations from both soils.

Table 8. Corn and soybean growth response (height and dry biomass) to 1.0 kg m⁻² of finely ground corn stover (CS) or high-lignin fermentation byproduct (HLFB) compared with not adding an amendment (control).

Treatment	Corn				Soybean, 60 DAP, growth chamber	
	66 DAP†, outdoors		60 DAP, greenhouse		Height	Biomass
	Height	Biomass	Height	Biomass		
Control	180.0 a‡	198.4 a	187 a	50.2 a	198 a	14.4 a
HLFB	177.5 ab	201.8 a	184 a	54.4 a	215 a	15.1 a
CS	164.2 b	144.0 b	181 a	48.0 a	192 a	14.8 a
LSD(<i>P</i> ≤ 0.05)	14.1	28.7	12	8.1	46	3.7

† Days after planting.

‡ Values in a column followed by a different letter are significantly different at *P* ≤ 0.05.

observed that it required almost threefold more HLFB amendment compared with corn stover to achieve a comparable amount of soil microbial biomass C, which also suggests reduced microbial activity when soil is amended with HLF. Because the HLF decomposes slower than corn stover (Table 5), we speculate that more C from HLF could be chemically or physically sequestered. The HLF was applied only once to soil with a relatively short incubation period. Related to the slow decomposition of lignin, it could take >118 d to observe all potential improvements in soil properties. In a field scenario, it is reasonable to expect repeated applications to achieve maximum benefits.

Plant Experiment

Soybean growth (height and biomass) was not affected by HLF (0.1 kg m⁻²) or corn stover amendment (Table 8). Corn grown outdoors had more biomass when amended with HLF than amending with corn stover, but did not differ from the control. Corn height was reduced by corn stover compared with the control, but not compared with the HLF-amended plants. There were no differences observed in corn plant height or biomass among the three treatments when corn was grown in the greenhouse. Inhibition of corn growth and yield when corn is grown continuously in rotation has been reported in several studies (Bhowmik and Doll, 1982; Crookston and Kurle, 1989). Yackle and Cruse (1983) suggested that autotoxic compounds were involved in causing this inhibition; however, Crookston and Kurle (1989) reported no effect of removal or addition of corn residue on the yield of either corn or soybean. Differences between our two corn experiments were attributed primarily to better light rather than differences in the potting mix, as neither water nor nutrients should have been limited. Care must be taken in attempting to extrapolate from our pot study to the field; however, our results suggest that HLF will have no negative impact on corn and soybean growth in the field if applied at ≤0.1 kg m⁻².

The National Renewable Energy Laboratory analyzed HLF to determine fermentation efficiency and energy value. The HLF is N enriched, compared with corn stover (Table 2), from microbial products used during fermentation. Growing crops in the presence of the material served as a direct, simple bioassay to screen for potential benefits or adverse reaction. Although this simple test is not a substitute for detailed chemical analysis, it did provide an inexpensive early screening tool. There are many aspects of potential environmental impacts of HLF that remain to be assessed, including evaluation for salts, acids, bases, organic compounds, etc., which have the potential to inhibit plant growth or soil processes or to have other negative environmental impacts.

The high rate of HLF needed to effect a change in soil properties was much greater than the rate tested for effects on plant growth. On sites with eroded or degraded soils, HLF should be applied at high rates and all corn stover should be returned to the soil rather than harvesting the stover for energy. Site application of the HLF may also be warranted since soils used in our study responded differently (cumulative CO₂ evolution and humic acid concentration) to the application of HLF. A positive crop response would be predicted if soil properties (e.g., water-holding characteristics or *D_b*) were improved by applying high rates or repeated applications of

HLF. A negative crop response to high or repeated application of HLF is feasible if a currently unidentified toxin (e.g., heavy metal) accumulates in the system. The current experiment was not designed to test this hypothesis. In addition, applying 10 kg m⁻² HLF with a C/N ratio of 30 (Table 2) has the potential to immobilize N, which could cause a period of insufficient plant-available N and potentially reduce plant growth or yield. These hypotheses need to be tested at the plot or field scale to develop application rate recommendations.

CONCLUSIONS

It is important to have environmentally and economically sustainable options for handling HLF before commercialization of cellulosic ethanol production. Several alternatives have been suggested for using HLF: HLF can be used for the production of heat and electricity (Sheehan et al., 2002, 2004), production of syngas (Ragauskas et al., 2006; Sricharoenchaikul et al., 2002), or application as an amendment to soil (Johnson et al., 2004). This study and our previous study (Johnson et al., 2004), suggest that HLF remaining after cellulosic ethanol production has potential value as a soil amendment. There are agronomic and economic questions that have not been addressed; for example, what is the recommended application rate for HLF or stover harvest?

The HLF can be added in sufficient quantities to evoke changes in soil properties, even in a soil with inherently high SOC. In both of our soils, humic acid concentration increased with higher rates of application of HLF. Application of the HLF, particularly at high rates, increased the number of water-stable aggregates and water retention in both soils. Increasing water-stable aggregates may reduce erosion risk (Skidmore and Siddoway, 1978). The slow decomposition of the HLF may be beneficial in retaining C in the soil. It takes considerably more HLF than corn stover, however, to achieve a comparable number of water-stable aggregates. Therefore, the amount of corn stover harvested for cellulosic ethanol production must be carefully weighed against using residue to protect the soil from erosion and loss of SOC. The Langhei soil, with a lower SOC, was more responsive to the addition of amendment than the Svea soil; this suggests selective placement of HLF for maximum benefit per unit applied.

These results demonstrate that HLF can enhance soil properties (e.g., *D_b*, water-retention characteristics, humic acid concentration, and water-stable aggregate percentage) and that its impact on these properties depends on concentration. When applied at a high rate (10 kg m⁻²), the impact on *D_b* and water retention was immediate and persisted for at least 118 d. At an application

rate of 0.1 kg m⁻² HLFB, which was an order of magnitude less than the concentration that demonstrated improved soil properties, there was no significant plant response to HLFB compared with the control. In one of our experiments, corn stover reduced corn biomass and height compared with the control. These observations warrant field-scale studies as we look for ways to maintain soil productivity while meeting the demands for a domestic, renewable energy—perhaps judicious application of the industrial HLFB to degraded soil with the remaining HLFB converted to heat or electricity. The developing cellulosic industry still faces many challenges and opportunities to design an industry that provides a domestic, renewable energy in a manner that sustains the underlying soil resource for future generations.

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