

# Characterization of Soil Amended with the By-Product of Corn Stover Fermentation

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## ABSTRACT

Corn (*Zea mays* L.) stover is a potential biofuel; however, removing this stover from the land may increase the risk of erosion and reduce soil organic matter (SOM). Land application of corn stover fermentation by-product, which is about 70% lignin, may reduce the environmental risk from biofuel harvest by helping to stabilize soil structure. A column study, with soil collected from a toeslope (noneroded, Svea; fine loamy, mixed, superactive, frigid Pachic Hapludoll) and a shoulder slope (severely eroded, Langhei; fine loamy, mixed, superactive, frigid Typic Eutrudepts) was conducted to evaluate the effect of fermentation by-product on soil properties. Soil was either not amended (control) or amended with corn stover or by-product at 0.75, 3.0, and 6.1 g kg<sup>-1</sup>. Soils were incubated for 123 d at ambient temperature in a laboratory, with an initial water-filled pore space (WFPS) of 0.6 m<sup>3</sup> m<sup>-3</sup> and drying cycles to 0.35 m<sup>3</sup> m<sup>-3</sup> WFPS. Compared with the control, amending soil with 6.1 g by-product kg<sup>-1</sup> increased CO<sub>2</sub> flux by 68% and increased soluble C and microbial biomass C by about 20%. In the severely eroded soil, humic acid concentration ( $r^2 = 0.97$ ,  $p = 0.009$ ) and aggregate stability ( $r^2 = 0.98$ ,  $p = 0.005$ ) increased linearly with increased by-product concentration. Water-holding capacity, bulk density, and aggregate distribution were not changed by soil amendments. Careful management of stover removal (avoiding eroded or erosion prone areas) and selective placement and rates of the by-product will contribute to a sustainable use of corn stover for ethanol production.

BURNING OF FOSSIL FUELS is a major contributor to atmospheric CO<sub>2</sub> (IPCC, 1996). As an alternative, the United States Department of Energy and private enterprise are developing a fermentation process for producing ethanol from high-cellulose biomass (Dipardo, 2000; Hettenhaus et al., 2000). Corn stover and other plant materials with a high concentration of cellulose have potential as biofuel (e.g., ethanol production). Use of biofuel may partially offset energy requirements currently fulfilled by fossil fuels (Paustian et al., 1998). A complete life-cycle analysis of this process includes comparing possible economical and environmentally sound uses for the by-product remaining after the fermentation of corn stover, such as production of electricity or use as a soil amendment.

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Corn stover harvested for ethanol production reduces the amount of residue returned to the soil. Crop residues on the soil surface are important in controlling wind and water erosion (Lindstrom, 1986). Removal of corn residue on both reduced tillage and no-till systems increased water runoff and soil erosion in the Northwestern Corn Belt of USA (Lindstrom, 1986). The amount of residue that can be removed without increasing the erosion risk is dependent on a soil's potential erodibility (Lindstrom and Holt, 1983). Lindstrom and Holt (1983) found that about 59% of residue was available for removal in all major land use areas, when the only criterion for removal was soil erosion. It should be noted that the amount removed varied from 0 to 100% depending on erosion risk (Lindstrom and Holt, 1983; Nelson, 2002). Recent reviews suggest that 20 (Nelson, 2002) to 30% (McAloon et al., 2000) of the total stover production could be made available for biofuel, based on ground cover requirements to control erosion.

Guidelines on how much residue can be removed from a given field still need to be resolved. In addition, data are still needed to determine whether managing for erosion is sufficient to maintain SOM levels (Wilhelm et al., 2003). Wilhelm et al. (2003) reported that contribution ratio of root (unharvestable material) to aboveground residue averaged 1.9, ranging from 0.8 to 2.6. Roots provide an important contribution to soil organic C (SOC), but aboveground residue also contributes to forming SOC. Clapp et al. (2000) found a decline in the mass of SOC at the end of 13 yr, if C inputs were limited to unharvestable C inputs for several tillage systems including no tillage.

Removal of corn stover also removes the valuable plant nutrients contained in the stover. Once removed, these nutrients are not cycled through the soil. Corn stover contains from 5.7 to 12.3 g N kg<sup>-1</sup> (Burgess et al., 2002; Green et al., 1995; Lindstrom and Holt, 1983; Mubarak et al., 2002; Natural Renewable Energy Laboratory [NREL], 2002), 1.8 to 2.9 g P kg<sup>-1</sup>, and 13.3 to 24.9 g K kg<sup>-1</sup> (Lindstrom and Holt, 1983; Mubarak et al., 2002). The by-product remaining after fermentation of corn stover contains 600 to 700 g lignin kg<sup>-1</sup> and 20 g N kg<sup>-1</sup> (NREL, 2002). In contrast, baled corn stover contains about 200 g lignin kg<sup>-1</sup> and 7 g N kg<sup>-1</sup> (NREL, 2002). The by-product of stover fermentation if applied to soil at the same rate would provide about three times as much lignin and N compared with stover.

Soil organic matter is important for many soil functions by providing energy, substrates, and biological diversity (Franzluebbers, 2002). Adams (1973) and Hudson (1994) found that a 2% decrease in SOM increased

**Abbreviations:** SOC, soil organic carbon; SOM, soil organic matter; WFPS, water-filled pore space; NREL, National Renewable Energy Laboratory, Golden, CO.

bulk density by  $0.1 \text{ Mg m}^{-3}$  or more. Water stability of soil aggregates is dependent on organic materials (e.g., polysaccharides, roots and fungal hyphae, persistent aromatic compounds; Tisdall and Oades, 1982). Soil organic matter affects soil compactibility, friability, soil water-holding capacity, air and water infiltration, nutrient conservation, and soil permeability (Carter, 2002). Improvement of soil aggregate stability results both from increased microbial activity utilizing carbohydrates and from the plant phenolics released during decomposition of structural components (e.g., lignin) (Martens, 2000).

Maintenance of SOM requires that efflux (decomposition of existing SOM) does not exceed influx of new C. Crop residue is an important source of new C for building and maintaining SOM. Buyanovsky and Wagner (1997) found that 80% of the crop residue added to a field is returned to the atmosphere within 2 yr. Corn residue C converted to SOM ranges from 8 to 18% (Barber, 1979). The small amount of new C that remained implies that a large C influx is important for maintaining SOM.

The underlying assumptions for this study were that residues are valuable for erosion control, organic matter input, and sustained productivity. The partial or complete removal of corn stover as a biofuel may increase the risk of erosion and/or accelerate SOM loss by reduced organic matter inputs. The degradation of the soil from erosion and the loss of SOM would reduce long-term productivity. It was hypothesized that since the by-product of stover fermentation was high in lignin, which is thought to play a role in stabilizing soil, soil incorporation of the by-product may help maintain or improve soil structure and stability. Therefore, application of fermentation by-product may reduce some of the negative effects of harvesting corn stover. The fermentation of corn stover for ethanol is still an experimental process. This is the first study to evaluate the impact of corn stover fermentation by-product on biological, chemical, and physical properties of two soils with contrasting organic C concentrations and erosion phases. The information from this experiment is part of the life-cycle analysis of using corn stover for ethanol production.

## MATERIALS AND METHODS

### Soils

Soil was collected from a complex soil association in an undulating landscape in west central Minnesota ( $45^{\circ}\text{N}$ ,  $96^{\circ}\text{W}$ ). Svea was the dominant soil series. Soils in the Svea series are well drained and moderately permeable with a very dark gray surface horizon, formed in calcareous glacial till. Langhei is a fine loamy, mixed, superactive, frigid Typic Eutrudepts. Five subsamples from each of these soils were collected for initial characterization (Table 1).

Soil was collected from the tilled layer (0–15 cm) at two sites in the same field. Svea soil was collected from the toeslope position (noneroded) and Langhei soil was collected from the shoulder slope (severely eroded). The field was in a soybean [*Glycine max* (L.) Merr.]–wheat [*Triticum aestivum* L.] rotation (4+ yr) with annual fall moldboard plowing, and spring

**Table 1. Initial soil characteristics of noneroded Svea and severely eroded Langhei soil.**

Characteristic	Noneroded	Severely Eroded
Sand, g kg	428	478
Silt, g kg	379	269
Clay, g kg	193	253
Total C, g kg <sup>-1</sup>	21.8	31.8
Organic C, g kg <sup>-1</sup>	20.4	3.0
pH(H <sub>2</sub> O)	7.9	8.3
pH(CaCl <sub>2</sub> )	7.4	7.8

(1 or 2) tillages. In early spring, bulk samples were collected from areas cropped to wheat the previous growing season. Soil was air-dried and passed through a 3-mm sieve.

### Treatments

The study had five treatments: no amendment (control); 2.4 g corn stover kg<sup>-1</sup> soil; and 0.75, 3.0, and 6.1 g by-product kg<sup>-1</sup> soil. The corn stover application rate was comparable with returning 4.3 Mg ha<sup>-1</sup> dry stover after grain harvest. The 0.75 g by-product kg<sup>-1</sup> soil had about the same amount of lignin as the corn stover treatment, while the 6.1 g by-product kg<sup>-1</sup> soil had comparable amounts of cellulose as found in the corn stover treatment (Table 2). By-product material and characteristics were provided courtesy of Jim McMillan at the NREL, Golden, CO. Air-dried and ground (4 mm) corn stover and by-product were added to about 1.47 kg soil, and then mixed thoroughly. The stover was ground to a size comparable with the by-product to prevent differences in decomposition due to particle-size differences.

Columns (polyvinyl chloride [PVC] cylinders, with sealed bottoms, 10 cm diameter, 20 cm height) were filled with soil and amendment mixture and packed to achieve an initial bulk density of  $1.3 \text{ Mg m}^{-3}$ . This corresponded to a total soil porosity of  $0.509 \text{ m}^3 \text{ m}^{-3}$ , assuming a particle density of  $2.65 \text{ Mg m}^{-3}$ . The experiment was initiated by wetting the columns to  $0.306 \text{ m}^3 \text{ m}^{-3}$  volumetric water, equivalent to  $0.60 \text{ m}^3 \text{ m}^{-3}$  WFPS. Maximum C mineralization with minimal denitrification occurs between 0.40 and  $0.60 \text{ m}^3 \text{ m}^{-3}$  (Franzluebbers, 1999; Doran et al., 1988; Linn and Doran, 1984). Water was allowed to evaporate from the soil to  $0.178 \text{ m}^3 \text{ m}^{-3}$  ( $\leq 0.35 \text{ m}^3 \text{ m}^{-3}$  WFPS). At that point water was added to return the soil to  $0.60 \text{ m}^3 \text{ m}^{-3}$  WFPS. The columns were incubated at ambient air temperature for 123 d in a building without air-conditioning. Average soil volumetric water and soil temperature are summarized in Fig. 1. Soil water did not differ between soils or among treatments.

### Analyses

Carbon dioxide flux was measured using a LI-COR CO<sub>2</sub>/H<sub>2</sub>O analyzer (Model LI-6262), a small soil chamber (Model 6000-09), a flow control unit (Model LI-670), and a data logger (LI-6200) according to the LI-COR Manual (Dugas, 1993; LI-COR Inc., 1990). A thin rubber gasket formed the seal between the soil chamber and the soil column. The soil chamber attached to the data logger was flushed with ambient air and placed over the soil column for data collection. After about 30 s, the flux was calculated from the rate of change of CO<sub>2</sub> concentration inside the chamber (LI-COR Inc., 1990). Fluxes were measured once daily the first 10 d of incubation and then less frequently as rates diminished. Measurements were taken between 0800 and 1200 h, rotating the sampling sequence to minimize potential diurnal flux bias. Perturbation by watering events typically resulted in temporary flux increases. The final measurements were taken on Day 118. Cumulative

**Table 2. Constituents and their concentration in organic materials used to amend soil.**

Constituent	By-product <sup>†</sup>	Corn stover <sup>‡</sup>
Lignin, g kg <sup>-1</sup>	624	200
Cellulose, g kg <sup>-1</sup>	125	355
Hemicellulose, g kg <sup>-1</sup>	28	230
Ash, g kg <sup>-1</sup>	168	116
Total C, g kg <sup>-1</sup>	486	466
N, g kg <sup>-1</sup>	20	7

<sup>†</sup> Composition of by-product provided courtesy of J. McMillan at NREL, Golden, CO.

<sup>‡</sup> NREL (2002).

flux as a function of time was calculated by assuming linearity between sampling points.

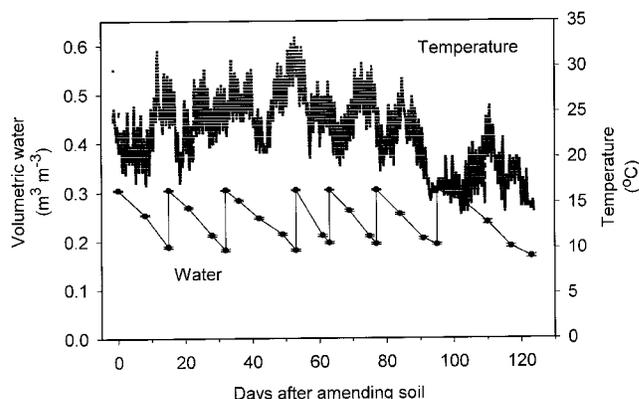
Soil was sampled at 1, 3, 7, 60, and 123 d following initiation for soluble C and microbial biomass C measurements. A small subsample was used to determine the moisture content at the time of sampling. The average gravimetric water content of the soil was 0.225 kg kg<sup>-1</sup> on Day 1, 0.214 kg kg<sup>-1</sup> on Day 3, 0.190 kg kg<sup>-1</sup> on Day 7, 0.160 kg kg<sup>-1</sup> on Day 60, and 0.124 kg kg<sup>-1</sup> on Day 123. Soluble C was extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub> from unfumigated (immediately after sampling) and after chloroform fumigation of the soil for 5 to 7 d. Microchemical oxygen demand tubes (20–900 mg L<sup>-1</sup> range) (Fisher Scientific, Pittsburgh, PA) were used for determining the concentration of soluble C. Dichromate reduction was measured as color development at 600 nm absorbance using a DU 7400 spectrophotometer (Beckman, Fullerton, CA). Glucose was used as a standard (Vance et al., 1987). Microbial biomass C was estimated by subtracting the soluble C in nonfumigated soil from the soluble C in a chloroform-fumigated soil and multiplying the difference by 2.64 (Vance et al., 1987).

Soil concentration of total N and total C 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 nitrate N concentration were measured using an Alpkem Autoanalyzer (Pulse Instruments Ltd., Saskatoon, SK), and pH<sub>(H<sub>2</sub>O)</sub> and pH<sub>(CaCl<sub>2</sub>)</sub> were measured using 2:1 ratio (Thomas, 1996).

The soil was fractionated into humin, humic acid, and fulvic acid fractions according to Stevenson (1994). The noneroded Svea soil had seven times greater organic C concentration compared with the severely eroded Langhei soil (Table 1). Therefore, 30 g of noneroded and 180 g of severely eroded soil were used to extract measurable quantities of humic acid. Soils were treated with 0.05 M HCl and washed with reverse-osmosis H<sub>2</sub>O to remove carbonates. This was a three times excess acid wash for removing carbonates in the noneroded Svea, but in the severely eroded soil, most of the C was inorganic, thus making it difficult to remove the carbonates. Presence of carbonates decreases the sensitivity of the LECO CN-2000 by increasing the likelihood of incomplete combustion.

After washing with HCl, the humic and fulvic acids were separated from the humin and inorganic fraction using repeated extractions with 0.5 M NaOH under N<sub>2</sub>. The humin fraction is the portion of humus that remains bound to the mineral soil after extraction with dilute alkali solution (Stevenson, 1994). The humin and humic fractions were freeze-dried. The dried fractions were ground and analyzed for total C and N and inorganic C. Due to the very high carbonate concentration in the severely eroded soil, inorganic C contamination was anticipated.

Before the initial wetting, preweighed stainless steel rings (5-cm i.d. by 5-cm deep) were inserted by hand into soil to measure water retention. The rings were positioned with the top of the ring at 1 cm below the soil surface. Three sets of



**Fig. 1. Average soil column temperature and volumetric soil water during experiment. The soil had a bulk density of 1.3 Mg m<sup>-3</sup>, and a porosity of 0.509 m<sup>3</sup> m<sup>-3</sup>; a volumetric soil water content of 0.306 m<sup>3</sup> m<sup>-3</sup> corresponds to a water-filled pore space of 0.60 m<sup>3</sup> m<sup>-3</sup>.**

columns had rings, one for each sample date (1, 60, and 123 d). Excess soil was trimmed level with the ends of each ring before placing the rings on a porous ceramic plate with a bubbling pressure of 0.1 MPa. The plate and soil within the rings were allowed to saturate overnight and then equilibrated at suctions of 0.01, 0.03, 0.05, and 0.1 MPa. Ring plus soil weights were obtained after equilibrating at each pressure. Following the 0.1 MPa measurements, soil dry weights were obtained after drying at 105°C for 24 h.

Aggregate stability and aggregate-size distribution were determined on termination of each experiment. A soil aliquot was removed from the column and air-dried before rotary sieving to measure aggregate-size distribution (Kemper and Rosenau, 1986). Aggregate stability of the 1- to 2-mm aggregates was determined in duplicate both on moist soil (water content at the time of sampling) and on soil material that had been air-dried and then remoistened in a humidified wetting chamber to near field capacity before wet-sieving at 40 strokes min<sup>-1</sup> for 5 min (Kemper and Rosenau, 1986).

### Statistical Analysis

The experimental design was a two by five factorial arrangement randomized within each of five replications. Within each replication were four identical columns of each soil-amendment combination treated as a statistical unit. The multiple columns per replication allowed for repeated destructive sampling. Parameters with multiple sample dates were analyzed for time by treatment or time by soil interactions, using a repeated measure analysis. Analyses were also done by day when time interactions were significant. Statistical differences were determined using generalized linear model,  $p \leq 0.05$  (Proc GLM; SAS version 8, SAS Institute Inc., Cary, NC).

Linear regression analysis (Proc REG; SAS) was used to investigate the relationships between by-product added and humic acid concentration, and between by-product added and aggregate stability on the severely eroded soil.

## RESULTS AND DISCUSSION

### Carbon Dynamics

#### Decomposition

Carbon dioxide flux is an indicator of decomposition and microbial activity. Amending with by-product or corn stover increased the flux and cumulative CO<sub>2</sub> released compared with the control by providing a C

**Table 3. Total CO<sub>2</sub> released from soil columns and the relative amount of C that originated from the amendment during incubation for 118 d.**

Soil	Amendment	Rate	Total accumulated CO <sub>2</sub>	Amendment C release‡
		g kg <sup>-1</sup>	g CO <sub>2</sub> m <sup>-2</sup>	%
Noneroded	Control	0.0	580 e§	NA¶
	By-product	0.75#	616 e	17 b
	By-product	3.0	785 d	15 b
	By-product	6.1	933 c	14 b
	Corn Stover	2.4††	1030 ab	45 a
Severely eroded	Control	0.0	590 e	NA
	By-product	0.75	724 d	46 a
	By-product	3.0	794 d	17 b
	By-product	6.1	1010 b	17 b
	Corn Stover	2.4	1080 a	51 a
Source of variance	df			
Soil (S)	1		**	**
Amendment (A)	4		†	†
S × A	4		NS‡‡	**

\*\* Significant at the 0.01 probability level.

† Significant at the 0.0001 probability level.

‡ Amendment C release = net C flux/g C in amendment × 100. Net C flux = (treatment flux - control flux).

§ Means within a column followed by a different letter are different at  $p = 0.05$ .

¶ NA, Not applicable.

# 0.75 g by-product kg<sup>-1</sup> soil had lignin equivalent to 2.4 g corn stover kg<sup>-1</sup> soil.

†† 2.4 g corn stover kg<sup>-1</sup> soil equivalent to 4.3 Mg ha<sup>-1</sup> dry stover after grain harvest.

‡‡ Not significant at the 0.05 probability level.

source on both soils (Table 3). Soil amended with corn stover released 1.8 times as much total CO<sub>2</sub> compared with the control. Compared with the control, the amount of CO<sub>2</sub> released from soil amended with by-product ranged from 1.1 times (0.75 g by-product kg<sup>-1</sup>) to 1.7 times (6.1 g by-product kg<sup>-1</sup>). Even with the 6.1 g by-product kg<sup>-1</sup> treatment, the by-product amended soil did not release as much CO<sub>2</sub> as the corn stover treatment.

The CO<sub>2</sub> flux on all sampling dates increased by amending the soil compared with the control (data not shown). The pattern of cumulative CO<sub>2</sub> released followed a double exponential equation ( $r^2 = 0.99$ ) for each soil and amendment combination. An exponential model provides a realistic model both mathematically and biologically (Wieder and Lang, 1982). Similar patterns of CO<sub>2</sub> released were reported by Ajwa and Tabatabai (1994), which they described with a multiple exponential function. The initial rate of decomposition

reflects initial concentration of N and readily available C, but as decomposition progresses, the rate reflects the decomposition of lignin (Berg and Matzner, 1997).

The observed increases in CO<sub>2</sub> fluxes (Table 3) after amending the soil with by-product or corn stover were associated with increased soluble C and increased microbial biomass C (Table 4). A soil by day interaction ( $p \leq 0.05$ ) was observed for both soluble C and microbial biomass C; therefore, separate analyses were conducted for each sampling date (1, 3, 7, 60, and 123 d; Table 4). A soil by amendment interaction ( $p \leq 0.05$ ) was not detected for soluble C or microbial biomass C, therefore only main effects are shown. The noneroded soil inherently had more soluble C and microbial biomass C compared with the severely eroded soil. Amending the soil increased both soluble C and microbial biomass C, but not equally among sampling dates. Soluble C and microbial biomass C for the 6.1 g by-product kg<sup>-1</sup>

**Table 4. Soil and amendment treatment means for soluble C and microbial biomass C sampled at several times during incubation with different levels of by-product or corn stover. Soil by amendment interaction was not significant at  $p \leq 0.05$ .**

Day	Soil		Amendment				
	Noneroded	Severely eroded	Control				Corn stover
			By-product		g kg <sup>-1</sup> soil		
				3.0	6.1	2.4‡	
Soluble C, µg C g <sup>-1</sup> soil							
1	83 a§	57 b	65 d	67 cd	72 ab	76 a	71 bc
3	78 a	57 b	62 b	63 b	66 b	73 a	74 a
7	73 a	59 b	62 b	61 b	68 a	70 a	69 a
60	69 a	53 b	56 c	56 c	61 bc	67 a	64 ab
123	55 a	44 b	45 c	47 bc	52 ab	54 a	49 ab
Microbial biomass C, µg C g <sup>-1</sup> soil							
1	366 a	277 b	310 a	306 a	331 a	338 a	324 a
3	403 a	349 b	334 c	354 c	384 b	411 a	398 ab
7	369 a	315 b	296 c	316 c	336 b	383 a	380 a
60	396 a	314 b	313 b	348 ab	368 a	377 a	367 a
123	334 a	250 b	260 c	284 bc	287 bc	329 a	301 ab

† 0.75 g by-product kg<sup>-1</sup> soil had lignin equivalent to 2.4 g corn stover kg<sup>-1</sup> soil.

‡ 2.4 g corn stover kg<sup>-1</sup> soil equivalent to 4.3 Mg ha<sup>-1</sup> dry stover after grain harvest.

§ Means within soil and amendment categories of each row followed by a different letter are different at  $p \leq 0.05$ .

soil and corn stover treatment were still greater than the control at the 123-d sampling date.

The amount of C released that originated from the newly added C compared with C previously in the soil was estimated based on net CO<sub>2</sub> flux (Table 3). The net CO<sub>2</sub> flux from a treatment was calculated by subtracting the amount of C released from the control treatment. This calculation neglects potential priming effects. Corn stover decomposed quicker and had up to 3.8 times more amendment C released than from the by-product treatments (Table 3).

The by-product had a C/N ratio of about 24 compared with about 70 for corn stover (Table 2; NREL, 2002). The lignin/N ratio was 31 for the by-product and 29 for corn stover. Based on C/N and lignin/N ratios, the by-product would be expected to decompose at the same or faster rate than corn stover. Other researchers (Gorissen and Cotrufo, 2000; Sowerby et al., 2000) also reported that C/N was inadequate for explaining decomposition. Decomposition rate is also dependent on N availability (Eiland et al., 2001a), WFPS (Doran et al., 1988), aeration, pH, reduction-oxidation potential, and temperature (Paul, 1991).

At the end of the four months, the soil columns released an average of 56% of the corn stover C and 20% of the by-product C. The differences in the C released from amendments reflect the differences in the decomposition rates of the component parts of the decomposing materials (Table 2). Half-lives are considered 0.6 d for sugars, 7 d for hemicellulose, 14 d for cellulose, and 364 d for lignin (Hagin and Amberger, 1974, cited in Kumar and Goh, 2000). Longer half-lives were reported for hemicellulose (21–26 d) and for cellulose (100–150 d) by Eiland et al. (2001b). Using Table 2 and literature range of reported half-lives, we predicted that about 50 to 60% of the corn stover should be decomposed by 118 d. Likewise, about 22 to 25% of the by-product should have decomposed. Thus, there was good agreement between the observed decomposition and the decomposition predicted based on decomposition of individual components (Tables 2 and 3).

The decomposition of corn stover was consistent with other laboratory incubations. Stott and Martin (1990) found that decomposing corn stover released 59% of initial C as CO<sub>2</sub> during 12 wk in a laboratory study. Burgess et al. (2002) found between 30 and 40% of corn stover in litterbags was decomposed during 193 d. Several researchers (Broder and Wagner, 1988; Burgess et al., 2002; Buyanovsky and Wagner, 1997; Stott and Martin, 1990) found that of the original C added in residue, only about 30% remained at 1 yr and only 10% remained at 2 yr from application. Added C remaining in the soil can contribute to SOM and may impact soil properties. Stott and Martin (1990) reported that at 1 yr, 20% of wheat lignin had decomposed with 99% of the remaining lignin associated with soil humus.

At the end of the study, 0.50 g C kg<sup>-1</sup> soil from corn stover remained in the soil, given that corn stover had 466 g C kg<sup>-1</sup> (Table 3). The by-product had 486 g C kg<sup>-1</sup>; at the 6.1 g by-product kg<sup>-1</sup> soil application rate, there would be 2.4 g C kg<sup>-1</sup> soil remaining at 123 d

(Table 2). The initial organic C concentration was 3 g C kg<sup>-1</sup> in the severely eroded soil and about 20 g C kg<sup>-1</sup> in the noneroded soil (Table 1). The C remaining in the noneroded soil from 2.4 g corn stover kg<sup>-1</sup> treatment represented only 2.5% of the initial SOC. In contrast, C from 2.4 g corn stover kg<sup>-1</sup> represented 17% of the organic C on the severely eroded soil. The C remaining after adding 6.1 g by-product kg<sup>-1</sup> to the soil represented 12% of the SOC on the noneroded soil and 80% of initial SOC on the severely eroded soil. Thus, it is reasonable to assume that added C impacted soil properties of the severely eroded soil more than it did those of the noneroded soil.

### Humins and Humic Fraction

Soil humus is stabilized SOM (Stevenson, 1994). Humins are organic matter bound tightly to the soil particles and remains associated with the mineral component of the soil (Stevenson, 1994). Amendment treatments did not alter the humin fraction concentrations in either soil (data not shown). In the severely eroded soil measurable inorganic C remained in the mineral component of the humin fraction.

Humic acid is defined as soluble in dilute alkali but insoluble in dilute acid (Stevenson, 1994). A soil by day interaction ( $p \leq 0.05$ ) was observed for humic acid and the C and N concentration in the humic fractions; therefore, separate analyses were conducted for each sampling date (1, 60, and 123 d; Table 5). A soil by amendment interaction ( $p \leq 0.05$ ) was not detected for the C and N concentration in the humic fractions, therefore only main effects are shown. The noneroded soil had 15 times greater humic acid, six to eight times greater C and about five times greater N in the humic fraction compared with the severely eroded soil. The C in the humic fraction was assumed organic. There was insufficient humic material to determine any inorganic C contamination. This was a crude extract and the low C and N concentrations were indicative of high ash content. It is common for humic acid extracted in dilute alkali and precipitated with dilute acid to contain inorganic contaminants (Stevenson, 1994).

Averaged across soils on Day 1, the by-product (even at the 0.75 g by-product kg<sup>-1</sup> rate) increased the concentration of humic acid and the C and N in the humic fraction compared with the control (Table 5). By 60 d, when averaged across soils, an increase in humic acid and C and N in the humic fraction were measurable only in the 3.0 and 6.1 g by-product kg<sup>-1</sup> treatments. By 123 d, the same pattern was still observed for humic C averaged across both soils, but not for humic N.

Humic acid concentration at 123 d increased on the severely eroded soil with addition of by-product ( $r^2 = 0.97$ ,  $p < 0.009$ ; Fig. 2). This relationship between humic acids and by-product was not apparent on the noneroded soil ( $r^2 < 0.1$ ;  $p > 0.05$ ). These results suggest that humification occurred for at least some of the amendment into the humic fraction. However, it is possible that the by-product was extracted from the soil during the humic acid extraction without it under going

**Table 5. Soil and amendment treatment means for humic acid, humic C, and humic N at different times during incubation with different levels of by-product or corn stover. Soil by amendment interaction was not significant at  $p \leq 0.05$ .**

Day	Noneroded	Severely eroded	Amendment				
			Control	By-product		Corn stover	
			0.0	g kg <sup>-1</sup> soil		g kg <sup>-1</sup> soil	
			0.0	0.75 <sup>†</sup>	3.0	6.1	2.4 <sup>‡</sup>
			Humic acid, g humic acid kg <sup>-1</sup> soil				
1	26 a§	2 b	12 b	14 a	15 a	16 a	13 ab
60	29 a	2 b	15 b	15 b	16 ab	18 a	15 b
123	36 a	2 b	20 a	18 a	20 a	21 a	18 a
			Humic C, g humic C kg <sup>-1</sup> soil				
1	4.6 a	0.9 b	2.2 d	2.6 cb	3.5 a	2.5 cd	
60	4.6 a	0.9 b	2.4 c	2.5 bc	2.8 b	3.4 a	2.4 c
123	4.8 a	0.8 b	2.6 bc	2.6 bc	3.1 ab	3.4 a	2.4 c
			Humic N, g humic N kg <sup>-1</sup> soil				
1	0.43 a	0.08 b	0.21 c	0.24 bc	0.27 ab	0.29 a	0.24 bc
60	0.43 a	0.08 b	0.24 b	0.24 b	0.26 b	0.29 a	0.24 b
123	0.44 a	0.08 b	0.25 a	0.24 a	0.28 a	0.29 a	0.23 a

<sup>†</sup> 0.75 g by-product kg<sup>-1</sup> soil had lignin equivalent to 2.4 g corn stover kg<sup>-1</sup> soil.

<sup>‡</sup> 2.4 g corn stover kg<sup>-1</sup> soil equivalent to 4.3 Mg ha<sup>-1</sup> dry stover after grain harvest.

§ Means within soil and amendment categories of each row followed by a different letter are different at  $p \leq 0.05$ .

humification. To test this possibility, the fermentation by-product and corn stover were subjected to the humic acid extraction procedure. After the procedure, at least 0.3 g g<sup>-1</sup> of the fermentation by-product was recovered in the humic fraction but only a trace of the corn stover was recovered in the humic fraction. From our present experiment it is not possible to ascertain the degree of humification, which occurred to the fermentation by-product, only that humic acid increased proportionally to by-product addition in an eroded soil.

### Nitrogen Dynamics

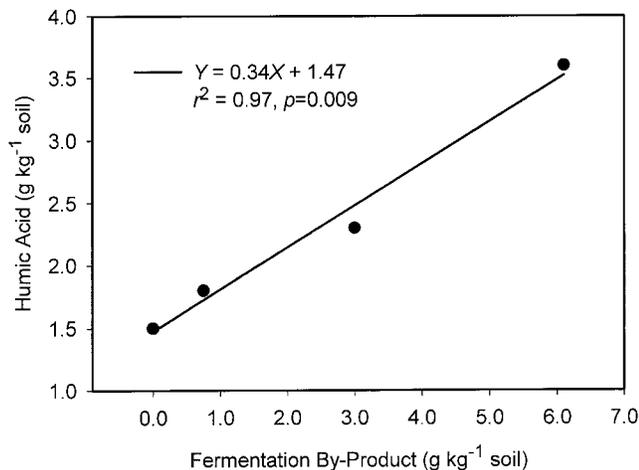
The addition of 3.0 and 6.1 g by-product kg<sup>-1</sup> of soil initially increased the concentration of total N compared with the control and the other amendment treatments (Table 6). Only the main treatment effects on total N are shown, as the soil by amendment interaction was not significant. By 60 d, differences in N concentration were no longer apparent among amendment treatments. A soil by day interaction was observed for NO<sub>3</sub><sup>-</sup>-N concentration. The NO<sub>3</sub><sup>-</sup>-N concentration increased in the

noneroded soil (48%) and severely eroded soil (74%) during the course of the experiment. The noneroded soil had about two times greater NO<sub>3</sub><sup>-</sup>-N compared with the severely eroded soil at each sampling date. Lower NO<sub>3</sub><sup>-</sup>-N concentration was detected in soil amended with corn stover at all three sample dates (1, 60, and 123 d) compared with the control or the by-product amended soils, probably due to greater immobilization due to high microbial activity.

Nitrogen mineralization was estimated from the difference between final and initial concentrations of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N (Tables 6 and 7). The production of NO<sub>3</sub><sup>-</sup>-N accounted for about 80% of the total N mineralized during the 123-d incubation in the by-product study. Soil amended with corn stover produced about three times less NO<sub>3</sub><sup>-</sup>-N (3.0 mg N kg<sup>-1</sup> soil) compared with the control and the by-product amended soils (average 9.5 mg N kg<sup>-1</sup> soil) during the first 60 d (Tables 6 and 7). The control treatment (16 mg N kg<sup>-1</sup> soil) produced 1.3 times greater NO<sub>3</sub><sup>-</sup>-N and the 6.1 g by-product kg<sup>-1</sup> soil treatment produced 1.6 times greater N (19 mg N kg<sup>-1</sup> soil), compared with corn stover (12 mg N kg<sup>-1</sup> soil) in 123 d. The NH<sub>4</sub><sup>+</sup>-N concentration (4.3 mg NH<sub>4</sub><sup>+</sup>-N kg<sup>-1</sup> soil) was not different between soils or among treatments at any sampling date.

Mineralization and immobilization of N during decomposition influences plant available N (Vigil and Kissel, 1991). The by-product had 20 g N kg<sup>-1</sup> and the corn stover had 7 g N kg<sup>-1</sup> (Table 2). Adding material with a N concentration <5 g kg<sup>-1</sup> to soil generally results in net immobilization (Vigil and Kissel, 1991). We found that net mineralization occurred, based on the increase in NO<sub>3</sub><sup>-</sup>-N with time (Tables 6 and 7). The amount of N mineralized from the added residue was highly variable among treatments with an overall coefficient of variation of 500%.

The addition of material with C/N ratio of 24 (by-product) or 70 (corn stover) (NREL, 2002) would be expected to cause at least initial N immobilization (Tisdall et al., 1986). Vigil and Kissel (1991) found that N



**Fig. 2. Concentration of humic acid as a linear function of by-product concentration 123 d after amending a severely eroded soil with the by-product following corn stover fermentation.**

**Table 6. Soil and amendment treatment means for total soil C ( $C_{tot}$ ), organic C ( $C_o$ ), mass ratio ( $C_o/N$ ) of organic C to total N, total N, and  $NO_3^-N$ . Soil by amendment interaction was not significant at  $p \leq 0.05$ .**

Property	Day	Soil		Amendment				
		Noneroded	Severely eroded	Control	By-product		Corn stover	
				g kg <sup>-1</sup> soil				
$C_{tot}$ , g kg <sup>-1</sup> soil	123	21.9 b§	30.1 a	0.0	0.75†	3.0	6.1	2.4‡
$C_o$ , g kg <sup>-1</sup> soil	123	19.8 a	4.1 b	25.7 b	25.7 b	26.2 b	26.9 a	25.3 b
$C_o/N$ ratio, g g <sup>-1</sup>	123	12.0 a	5.6 b	11.3 ab	10.4 b	12.8 ab	13.6 a	11.5 b
Total N, g kg <sup>-1</sup>	1	1.64 a	0.67 b	7.9 a	8.8 a	9.6 a	10.3 a	7.3 a
$NO_3^-N$ , mg kg <sup>-1</sup>	60	1.71 a	0.73 b	1.1 b	1.1 b	1.2 a	1.2 a	1.1 b
	123	1.72 a	0.69 b	1.2 a	1.2 a	1.2 a	1.2 a	1.2 a
	1	37.3 a	18.4 b	1.2 a	1.2 a	1.2 a	1.3 a	1.2 a
	60	47.7 a	24.4 b	29.3 a	28.6 a	30.1 a	29.5 a	21.8 b
	123	55.1 a	31.9 b	38.5 a	39.4 a	38.6 a	38.9 a	24.8 b
	1			45.5 ab	46.3 ab	43.7 b	48.1 a	33.9 c

† 0.75 g by-product kg<sup>-1</sup> soil had lignin equivalent to 2.4 g corn stover kg<sup>-1</sup> soil.

‡ 2.4 g corn stover kg<sup>-1</sup> soil equivalent to 4.3 Mg ha<sup>-1</sup> dry stover after grain harvest.

§ Means within soil and amendment categories of each row followed by a different letter are different at  $p \leq 0.05$ .

and the lignin to N ratio could explain 80% of the variability of N mineralized in a season from incorporated crop residue. Using their equation, [% N mineralized =  $0.62 + (1.333 \times \text{g N kg}^{-1}) - (0.875 \times \text{lignin/N ratio})$ ], we predicted that 9% of the corn stover N and 27% of the N from the by-product should be mineralized, which overestimated the amount of mineralization observed (Tables 6 and 7). When using the Vigil and Kissel (1991) equation based on the N concentration and lignin concentration [N mineralized =  $-5.01 + (1.52 \times \text{g N kg}^{-1}) - (0.004 \times \text{g lignin kg}^{-1})$ ], the predicted value was nearer our observed amounts of N mineralized.

In our experiment, the ratio of observed C released to N mineralized ( $C_{rel}/N_{min}$ ) increased with the addition of by-product or corn stover (Table 7) and exceeded the C/N ratio of the by-product and corn stover. Nitrogen immobilized into the microbial biomass was likely the reason for increasing  $C_{rel}/N_{min}$ . Corn stover addition led to immobilization of N into microbial biomass (Table 7). Soil amended with 6.1 g by-product kg<sup>-1</sup> soil or with the corn stover, had a larger microbial biomass C pool compared with the control at all sampling dates except the initial sample date (Table 4). As the amount of readily available C (e.g., cellulose and hemicellulose) increases, C and N incorporated into structural components of microbial biomass should also increase.

### Aggregate Stability

The severely eroded soil had 596 g water-stable aggregates kg<sup>-1</sup> compared with 540 g water-stable aggregates kg<sup>-1</sup> measured on the noneroded soil samples before air-drying, but after air-drying there were 805 and 868 g water-stable aggregates kg<sup>-1</sup> in the severely eroded and noneroded soils, respectively. Air-drying before measuring aggregate stability has been previously shown to increase the water-stable aggregate fraction (Gollany et al., 1991).

A significant soil by amendment interaction was observed for aggregate stability measured only on air-dried samples. Aggregate stability of the severely eroded soil increased with additions of by-product ( $r^2 = 0.98$ ;  $p < 0.005$ ; Fig. 3). Soil amended with corn stover had 810 g water-stable aggregates kg<sup>-1</sup> compared with 840 g water-stable aggregates kg<sup>-1</sup> in soil amended with 6.1 g by-product kg<sup>-1</sup>. There were no measurable changes in water-stable aggregates on the noneroded soil associated with amending the soil with fermentation by-product or corn stover compared with the control.

The severely eroded soil averaged 4.1 g organic C kg<sup>-1</sup> averaged across amendment treatments, 123 d after application (Table 6), a 37% increase compared with initial concentration (Table 1). The organic C concentration was the same on the noneroded soil before and

**Table 7. Mass ratio of C released to N mineralized ( $C_{rel}/N_{min}$ ), net N mineralization, N immobilized, and fraction of net N mineralized from amendment during the 123-d incubation with different level of by-product or corn stover.**

Observed or Predicted	Soil		Amendment				
	Noneroded	Severely eroded	Control	By-product		Corn stover	
				g kg <sup>-1</sup> soil			
$C_{rel}/N_{min}$ , g g <sup>-1</sup>	60 b§	78 a	0	0.75†	3.0	6.1	2.4‡
Predicted C, g kg <sup>-1</sup> soil	0.19 a	0.14b	0.18 a	0.15 a	0.17 a	0.17 a	0.14 a
Actual minus predicted, g C kg <sup>-1</sup> soil	0.82 b	0.91a	0.55 e	0.69 d	0.86 c	1.04 b	1.19 a
Net total N mineralization, $\mu\text{g N g}^{-1}$ soil¶	18 a	14 b	18 a	18 a	12 a	18 a	13 a
N immobilized, g N kg <sup>-1</sup> soil	0.14 a	0.13 a	0.09 a	0.15 a	0.10 a	0.016 a	0.16 a
N Mineralized from amendment N, g g <sup>-1</sup> #	0.04 a	-0.11 a	NA††	0.13 a	-0.06 a	0.04 a	-0.23 a

† 0.75 g by-product kg<sup>-1</sup> soil had lignin equivalent to 2.4 g corn stover kg<sup>-1</sup> soil.

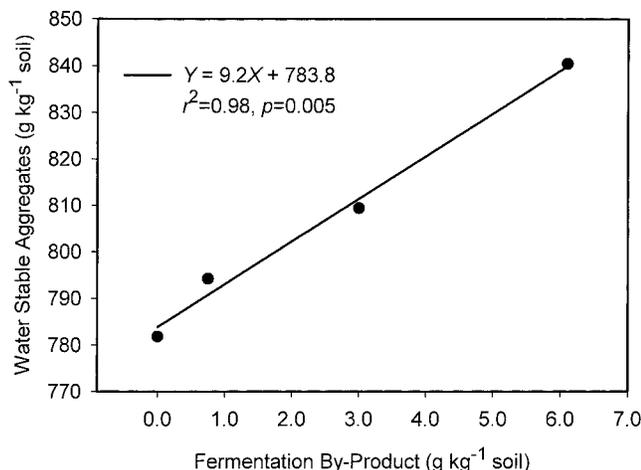
‡ 2.4 g corn stover kg<sup>-1</sup> soil equivalent to 4.3 Mg ha<sup>-1</sup> dry stover after grain harvest.

§ Means within soil and amendment categories of each row followed by a different letter are different at  $p \leq 0.05$ .

¶ Total N mineralized in the amended sample minus the N mineralized in the control, divided by the N added in the amendment.

# A negative value indicates immobilization.

†† NA, not applicable.



**Fig. 3.** Water-stable aggregates from air-dried severely eroded soil as a linear function of by-product concentration 123 d after amending a severely eroded soil with the by-product following corn stover fermentation.

after amendments (Tables 1 and 6). On the severely eroded soil, humic acid concentration, and stable aggregates increased linearly with increased by-product addition (Fig. 2 and 3). Increased organic matter tends to increase water-stable aggregation (Tisdall and Oades, 1982). These results provide limited evidence that the application of the fermentation by-product would have a positive influence on soil stability at least on a degraded soil.

### CONCLUSIONS

This work shows that amending soil with the fermentation by-product following ethanol production from corn stover could improve soil properties. However, returning by-product to the field may not negate all potential problems of removing corn stover, as it provides little ground cover. Amending the soil with by-product adds slowly decomposing C to the soil. The addition of new C may have a larger impact on soil properties of severely eroded soil with low organic C compared with noneroded soil with a large pool of organic C. The slow decomposition and long residence time of the by-product in the soil may allow the by-product to make a large and long-term contribution to SOM, which could be especially beneficial in severely eroded soils.

Corn stover is not a waste product. Corn stover provides a food source for soil fauna, surface residue to minimize erosion, and contributes to nutrient cycling (e.g., C, N, P). Thus, sustainable use of corn stover for ethanol production could be developed only with prudent management of stover removal (avoiding highly eroded areas and assuring maintenance of SOC) and selective placement of the by-product (spreading it on eroded knolls).

Many issues remain concerning the overall feasibility of using corn stover or other high cellulose biomass for ethanol production. While fermentation processes advance, the cost of removing corn stover and its environmental contributions should not be ignored. Before

stover removal becomes widespread, soil-specific removal rates should be determined for profitable stover use with minimal erosion, nutrient, and SOM losses. Application of fermentation product to eroded soils may provide both a safe means of disposal as well as a benefit to soil properties.

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