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Assessing changes in soil microbial communities and carbon mineralization in Bt and non-Bt corn residue-amended soils

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ABSTRACT

The effects of Bt corn (*Zea mays* L.) residue on soil microbial communities and rates of C mineralization were investigated. The Bt corn residue had a higher lignin content (12%) and lignin/N (9.9) ratio compared with its non-Bt near-isoline (10% lignin; lignin/N = 8.6). We examined the relationships among the Bt/non-Bt residue properties, residue component, soil texture, sampling time, and tillage management in microcosm and field studies. Bt corn residue incorporated in soils of different textural classes (silty clay, silt loam and sandy loam) in microcosms affected bacterial substrate metabolism. Substrate utilization profiles (Biolog) of soils amended with Bt residue differed from those with non-Bt residue based on principal component analysis (PCA). Denaturing gradient gel electrophoresis (DGGE) patterns revealed only slightly altered microbial communities in the soils amended with Bt residue compared with the non-Bt isoline. Soil texture significantly ($P < 0.05$) affected C mineralization and substrate utilization profiles. Carbon dioxide evolution rate constants (k) of 0.085–0.087 for non-Bt and Bt corn leaf tissue added to silt loam indicated higher rates of soil CO_2 evolution compared with addition of roots and stems ($k = 0.06$–0.07). However, cumulative CO_2 production after 73 days was similar regardless of residue component amendment. Significant ($P < 0.05$) interactions between soil texture, residue type (Bt versus non-Bt) and residue component illustrated the influence of soil on decomposition. In the field study, sampling time significantly correlated with Biolog metabolic activity and DGGE profiles. The field study also confirmed the effects of Bt residue on total plate count and substrate utilization profiles. Based on the results of the microcosm and field studies, we concluded that incorporation of Bt residue with higher lignin content and lignin/N ratio in soil significantly affected the structure of microbial communities compared with the residue from its non-Bt isoline. Abiotic factors including soil texture and sampling time also influenced the soil microbial communities and the decomposition of corn residues.

1. Introduction

Transgenic corn hybrids expressing insecticidal proteins derived from *Bacillus thuringiensis* (Bt) have several advantages for crop production, including increased insect resistance, reduced insecticide application, increased grain yields and other improvements in plant growth (Graeber et al., 1999; Lauer and Wedberg, 1999; Obrycki et al., 2001). However,

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reductions in the damage from European corn borer (*Ostrinia nubilalis*, Hübner) or differences in the amount and composition of Bt corn residues may increase the amount of residues remaining on or in the soil and affect mineralization of C and nutrients (Motavalli et al., 2004). Advantages of leaving higher densities of crop residues in the field include increased inputs of organic materials that benefit soil biological and biochemical properties and overall soil and water conservation (Unger, 1994; Schomberg et al., 1994; Mullen et al., 1998; Padgitt et al., 2000).

Soil microorganisms are critically important in the decomposition of crop residues. The physical and chemical nature of plant materials influences microbial decomposition (Collins et al., 1990). The use of cover crops generally increased soil organic C and stimulated bacterial growth and activity (Bolton et al., 1985; Kirchner et al., 1993; Mullen et al., 1998). Knowledge of the impact of transgenic crop residues on soil microbial ecology is essential for understanding the long-term agronomic and environmental effects of genetically modified crops and for developing appropriate management practices for minimizing potential negative impacts.

Differences in the environmental fate of crop residues from transgenic Bt crops compared with non-transgenic crops may occur through several mechanisms, including: (i) alterations in C mineralization rates due to the differences in the composition, quantity, and physical form of Bt corn residues compared with residues from non-transgenic varieties; (ii) inhibition or stimulation of soil microbial communities, possibly through released chemical compounds in the residues (e.g., Bt toxin) or other differences in the amount and composition of the Bt residues and (iii) changes in management practices (e.g., reduced pesticide applications) that may occur because of the use of the Bt crop (Motavalli et al., 2004).

Several studies have reported various effects of Bt endotoxins released in root exudates, and from biomass and residues on soil microorganisms. Generally, insecticidal toxin released from Bt crops had no short-term deleterious effects on soil biological communities, but the potential long-term effects due to accumulation and persistence of the toxin on soil biodiversity have not been evaluated extensively (Donegan et al., 1995; Betz et al., 2000; Saxena and Stotzky, 2001a; Head et al., 2002; Zwahlen et al., 2003). Research on the effects of altered chemical or physical properties of Bt corn residues on decomposition have yielded conflicting results. Those transgenic Bt corn hybrids with higher lignin contents compared with non-transgenic corn may lead to slower residue decomposition by soil microorganisms (Masoero et al., 1999; Saxena and Stotzky, 2001b). In contrast, Bt corn varieties with low lignin content, low C/N, and high soluble carbohydrate content in leaves are documented (Escher et al., 2000). Therefore, further study is required to fully understand the properties of transgenic crop residues and the effects on microbial activity including decomposition.

Microbial communities in soils are typically assessed by using culture-based methods, which detect only a small proportion of soil microorganisms (Liesack et al., 1997). Culture-independent assays, such as denaturing gradient gel electrophoresis (DGGE), are more sensitive for assessing a broader range of microbial diversity (Muyzer et al., 1993; Heuer et al., 2002). In this research, the microbial structure and activity in soil amended with residues of Bt corn and its non-transgenic near-isoline were evaluated by PCR-DGGE in combination with Biolog substrate utilization and total plate counts (Garland and Mills, 1991; Muyzer et al., 1993). The use of these phenotypic and genotypic methods may yield a more accurate assessment of the impacts of transgenic corn residues on soil bacterial communities. Previous studies showed that bacterial communities differed among soils of contrasting textures suggesting that microbial activity likely differs as well (Fang et al., 2005). Soil management including tillage also affects residue decomposition because incorporation in soil often leads to more rapid decomposition than in no-tillage systems in which residues remain on the soil surface (Mungai et al., 2005). The objective of this study was to investigate and compare the effects of Bt and non-Bt corn residue amendments on soil microbial communities and rates of C mineralization in soils with varying texture and tillage. This study focused on the effects of transgenic and non-transgenic corn residues on the overall microbial community rather than on specific cellulose- and lignin-decomposing microorganisms, which will be examined in subsequent studies.

2. Materials and methods

2.1. Field study

Bt corn (Merschman M-0012Bt[1]; Merschman Seeds, West Point, IA) and its non-Bt near-isoline (Merschman M-00110) were no-tilled planted with four other Bt varieties and their respective isolines at a rate of 61,750 seeds ha^{-1} in April 2002 in a split block design with four replications at the Greenley Agricultural Experiment Station (40°02′N, 92°14′W) in Northeastern Missouri. Complete details of all corn varieties are presented in Mungai et al. (2005). Plots measured 3.0 m wide by 10.6 m long with 76-cm row widths. Only information regarding the corn hybrid Merschman M-0012Bt and its non-transgenic isoline Merschman M-00110, which were phenotypically identical, will be presented. The soil at the site was classified as a Mexico silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualfs); selected soil properties are presented in Table 1. Plots were fertilized with 24 kg P ha^{-1} and 93 kg K ha^{-1} in fall 2001. Nitrogen was applied as anhydrous ammonia in the spring of 2002 at a rate of 202 kg N ha^{-1}. Plots were maintained weed-free with a preemergence application of atrazine/metolacholor at 3.9 kg ha^{-1} plus simazine at 560 g ha^{-1} followed by bromoxynil applied postemergence at 280 g ha^{-1}.

Fifteen whole corn plants were harvested per plot in October 2002 and weighed to determine total above-ground biomass on a dry weight basis. Harvested plants were separated to determine stem and leaf weights of remaining aboveground plant material that would be returned to the surface of each plot as crop residues after grain harvest. Roots

[1] Trade names are for clarity of information and do not represent endorsement by the USDA-ARS or by the University of Missouri.

Table 1 – Selected physical and chemical properties for the field soil and for soils used in the microcosms

Soil textural class	Soil textural analysis			pH (0.01 M $CaCl_2$)	Total organic C (%)	Total N (%)	Bray-1 P (mg kg^{-1})	Exchangeable		
	Sand (%)	Silt (%)	Clay (%)					Ca (mg kg^{-1})	Mg (mg kg^{-1})	K (mg kg^{-1})
Field										
Silt loam	22.3 (1.0)[a]	72.8 (1.0)	4.9 (0.1)	5.4 (0.1)	2.2 (0.3)	0.15 (0.01)	18 (3)	2438 (90)	395 (46)	157 (3)
Microcosms										
Silt loam	12.5 (0.1)	70.0 (0.1)	17.5 (0.1)	6.7 (0.1)	3.5 (0.2)	0.21 (0.01)	34 (2)	3805 (204)	372 (20)	236 (14)
Sandy loam	67.5 (0.1)	22.5 (0.1)	10.0 (0.1)	7.6 (0.1)	0.6 (0.2)	0.07 (0.01)	23 (1)	2873 (14)	132 (2)	123 (5)
Silty clay	15.0 (0.1)	45.0 (0.1)	40.0 (0.1)	6.7 (0.8)	1.6 (0.1)	0.13 (0.01)	3 (1)	4955 (880)	541 (114)	168 (8)

[a] S.D. is shown in parenthesis.

were also collected from five plants per plot. All collected corn plant components were dried at 60 °C to determine moisture and then ground in a stainless steel Wiley Mill to pass a 1 mm screen. Stem, leaf and root components were analyzed for total organic C (Nelson and Sommers, 1975), total N (Zellweger Analytics, 1996), and lignin and ash content (Goering and VanSoest, 1970).

After grain harvest and surface application of remaining plant residues, plots were split into a block that remained in no-till and a block that was disk-harrowed (5–8 cm depth of incorporation) in November 2002. Soybean 'Asgrow 3701' (Roundup Ready®) was planted at a rate of 445,000 seeds ha^{-1} on 1 June 2003. Glyphosate at 840 g ha^{-1} was applied as burndown and postemergence treatments on 20 May and 13 June, respectively.

Beginning in October 2002, 15 soil subsamples were collected per plot to a depth of 15 cm using a stainless steel push probe and composited into a single sample. Additional samples were taken in May, July, and October 2003. The soil samples were immediately placed in a cooler with ice packs and stored at 4 °C before analysis.

2.2. *Microcosm study*

The potential interactive effects of Bt corn residues and soil texture on C mineralization rates were determined in microcosms using the aerobic leaching method described by Motavalli et al. (1994, 1995). Briefly, microcosms consist of 150-ml Falcon filter units fitted with 0.2-μm cellulose acetate membrane filters and glass fiber pre-filters onto which soil (50 g) is placed and covered with a glass microfiber filter. The microcosms were leached under suction to simulate water movement under field conditions. Soils representing three textural classes (silt loam, silty clay and sandy loam) were collected from the 0–20 cm depth at cultivated field sites in central Missouri. Silt loam was collected from the Ap horizon of a Mexico silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualfs); silty clay soil collected from the exposed argillic horizon of a Mexico soil from which the Ap horizon was physically removed >5 years prior to sampling for an erosion experiment; and a sandy loam was collected from the Ap horizon of a Haynie sandy loam (coarse-silty, mixed, calcareous, mesic Mollic Udifluvents). All soils originated from field sites managed under similar corn–soybean rotations as detailed in Fang et al. (2005).

The soils were air-dried and ground to pass a 2 mm sieve. The soils were analyzed for pH (1:1 (w/v), 0.01 M $CaCl_2$), total organic C (Nelson and Sommers, 1975), soil test Bray-1 P (Bray and Kurtz, 1945), exchangeable Ca^{2+}, Mg^{2+}, and K^+ extracted with 1 M ammonium acetate at pH 7.0 (Warncke and Brown, 1998) and soil texture by the pipette method (Gee and Bauder, 1986). Dried and ground corn root, leaf and stem components of the Bt and non-Bt corn residues were added to the soils at a rate of 2 mg g^{-1} soil and the amended soils placed in the microcosms. An additional set of unamended soils were included as controls. Microcosms were arranged in a completely randomized design with four replications and maintained in a constant temperature room at 25 °C in the dark with a constant soil water potential of −47 kPa. Soil CO_2 efflux was measured at 3, 7, 15, 22, 28, 43, 58 and 73 days after the

start of the incubation in the sealed head space of the microcosms using a gas chromatograph (Buck Scientific, Norwalk, CT) equipped with a thermal conductivity detector (TCD). At the end of the incubation, soil samples were removed from the microcosms and analyzed for culturable bacteria (Zuberer, 1994), substrate utilization patterns (Garland and Mills, 1991), and PCR-DGGE (Muyzer et al., 1993).

2.3. Soil microbial community determinations

2.3.1. Carbon substrate utilization

Biolog GN2 microplates (Biolog Inc., Hayward, CA) were used to analyze substrate utilization patterns of soil microbial communities of soil collected from the field and microcosm experiments. The usefulness of the Biolog system for providing a meaningful insight into the functional ability of bacterial communities compared across different soils has been documented (Preston-Mafham et al., 2002). Soils were suspended in 0.85% NaCl and 10-fold serially diluted. Microplates were inoculated with 125-μL aliquots of the 10^{-3} dilution. After the plates were incubated at 25 °C for 72 h, optical density (OD) of the wells was measured using a microplate reader (Dynatech MR 5000, Chantilly, VA) at 575 nm. Overall color development in Biolog plates was expressed as average well color development (AWCD) (Garland and Mills, 1991). The substrate-utilization patterns were subjected to principal component analysis (PCA) using AWCD as a covariable. After inoculation of Biolog plates, the soil suspensions were analyzed for culturable bacteria using Tryptic Soy Agar (TSA; Sigma, St. Louis, MO) supplemented with 80 mg g^{-1} of cycloheximide to suppress fungal growth. This medium tends to select for copiotrophic bacteria, which were assumed to be most affected by soil amendments that in the short-term disrupt readily available substrates that are metabolized by this group, thus serving as a general indicator of immediate effects on soil microbial activity. Plates were incubated at 27 °C for 3 days, after which bacterial colonies were enumerated.

2.3.2. PCR-DGGE

Total soil DNA was extracted using the Soil Isolation DNA Kit (MoBio Laboratories, Solana Beach, CA). The extracted DNA was quantified by absorbency measurement using a spectrophotometer at 260 nm and stored at −20 °C. Universal bacterial primers (F984GC-R1378) targeting 16S rDNA at 968–1401 bp (*E. coli* rDNA sequence) were used for PCR (Heuer and Smalla, 1997). The PCR mixtures had a final volume of 50 μL and contained 20 pmol of each primer and 10–25 ng DNA template in 2× Red TaqReadyMix (Sigma-Aldrich Co., St. Louis, MO). PCR was performed in an Eppendorf Mastercycler Thermal Cycler (Perkin-Elmer, Norwalk, CT) using the following program: 94 °C for 4 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min and a final extension at 72 °C for 10 min, then held at 4 °C. The resulting PCR products were analyzed on a 1% agarose gel to confirm their size and yield.

Denaturing gradient gel electrophoresis was performed using a DCode Universal Mutation Detection System (BioRad, Hercules, CA) on a polyacrylamide gel (8%) with a gradient of 35–55% denaturant (urea + formamide) at 160 V for 6 h. DNA bands in the gels were stained with SYBR Green I (Molecular Probes, Eugene, OR) and patterns were digitized and photographed by using the GeneGenius Gel Documentation System (Syngene, Frederick, MD) and Genetool software (Syngene). Quantification of band intensities with this system aided in interpreting differences between gel patterns from the various soil treatments.

2.4. Statistical analysis

Soil CO_2 efflux, cumulative CO_2 evolved, AWCD from Biolog metabolic profiles, and culturable bacteria populations (after log transformation) were normally distributed and were subjected to the stepwise multivariate regression analysis. We assigned 0 for non-Bt and 1 for Bt and the same designation for no-tillage and tillage. For the hierarchical variables, we made sandy loam = 1, silt loam = 2 and silty clay = 3, and for the plant materials we designated root = 1, stem = 2 and leaves = 3. Comparison among means of these parameters was also carried out using the Fisher's protected least significant difference (LSD) test in one-way ANOVA at $P \leq 0.05$.

To compare the difference of carbon-sources among the different groups, the PC-test (Glimm et al., 1997) was employed because the conventional test methods could not be addressed due to the high-dimensional observations with the small sample size. The PC-test is based on the assumption that a few latent factors underlie any difference in the carbon-source consumption among the 96 wells. The latent factors can be determined by a principle component analysis (PCA) on the basis of the covariance matrix. Based on the PCA, we chose, the first k principle components, of which the cumulative contribution was ≥75%, as the latent factors. Läuter (1996) demonstrated that the PC-test attains highest power while maintaining the exact α-level. Thus, if the PC-test yields significance, it indicates there is a significant difference between the two communities. Cluster analyses of DGGE profiles were based on the position of DNA bands using binary values (0 for an absent band and 1 for a visible band). The similarity indices of DGGE patterns were determined by group average (unweighted pair group with mathematical averages, UPGMA) using the SAS statistical program (SAS Institute, 1988).

3. Results

3.1. Residue inputs and cover in field study

Among the five Bt corn varieties and their respective non-Bt isolines grown during 2002, Merschman M-0012Bt and Merschman M-00110 were the only pair of varieties which showed significantly higher lignin content and lignin/N ratio in the Bt compared with the non-Bt variety (Table 2, data not shown for other varieties). Therefore, these isolines were selected to study the effects on soil microbial communities and soil C mineralization. The average (±standard deviation) above-ground biomass for the Merschman non-Bt and Bt varieties at harvest were 9.91 ± 4.16 and 14.59 ± 3.32 Mg ha^{-1}, respectively. Differences in biomass yields were primarily due to greater stem damage from heavy European corn borer (*O. nubilalis*, Hübner) infestations in the non-Bt corn during 2002 (Mungai et al., 2005).

Table 2 – Selected chemical characteristics of the Bt and non-Bt plant residues from the field study used in the microcosms

Residue component	Variety	Total organic C (%)	Lignin (%)	Ash (%)	Total N (%)	C/N	Lignin/N
Root	Bt	43.5 (0.09)[a]	11.7 (0.12)	17.3 (0.18)	1.20 (0.01)	36.7 (0.14)	9.89 (0.16)
	Non-Bt	41.7 (0.15)	9.94 (0.02)	20.8 (0.28)	1.15 (0.01)	36.3 (0.13)	8.64 (0.02)
Stem	Bt	50.6 (0.01)	7.75 (0.01)	3.25 (0.07)	0.52 (0.01)	97.4 (0.03)	11.8 (0.41)
	Non-Bt	49.8 (1.56)	4.30 (0.01)	3.79 (0.02)	0.66 (0.01)	76.0 (1.57)	8.27 (0.03)
Leaf	Bt	48.9 (0.08)	3.29 (0.02)	7.11 (0.15)	0.71 (0.01)	68.9 (0.11)	4.63 (0.03)
	Non-Bt	48.3 (0.04)	2.10 (0.01)	8.25 (0.07)	0.96 (0.01)	50.6 (0.33)	2.20 (0.01)

[a] S.D. is shown in parenthesis.

Stem and leaf weights were not significantly different between Bt and non-Bt varieties. Total stem plus leaf yield for the non-Bt variety was 4.02 ± 1.38 Mg ha^{-1} compared with 5.47 ± 1.36 Mg ha^{-1} for the Bt variety. Tillage practice caused the greatest differences in residue cover; variety differences had no effect on amount of residue cover. Residue cover in the no-till plots averaged $35.4 \pm 13.3\%$ for the non-Bt and $33.9 \pm 2.7\%$ for the Bt variety. The proportion of residue cover was lower on the tilled plots, averaging $26.3 \pm 6.1\%$ for the non-Bt and $29.4 \pm 11.6\%$ for the Bt variety.

3.2. *Characterization of plant tissue and soils*

The C/N, lignin/N, lignin and ash contents of individual plant components varied widely (Table 2). The Bt residues, including root, stem and leaves had higher lignin, lignin/N ratio and total organic C contents than those of non-Bt residues. Total N was lower in Bt stem and leaf of but higher in roots compared with the non-Bt isoline. Roots also contained higher lignin, but lower C/N and total organic C content than those of other components.

For soils used in the microcosms, the sandy loam contained lower levels of total organic C, total N, and exchangeable Ca, Mg and K compared with both silt loam and silty clay (Table 1). The silty clay had the lowest Bray-1 P content among the three soils. The initial soil pH, total organic C, total N, Bray-1 P and exchangeable Ca, Mg, and K of the silt loam in the field study were generally lower than the silt loam used in the microcosms.

3.3. *Soil respiration*

Efflux of CO_2 did not differ for any individual residue component incubated in the soil microcosms, therefore, measurements were pooled over components and mean CO_2 efflux from combined residues were determined for each soil (Fig. 1). Soil CO_2 efflux generally decreased over time regardless of corn residue amendment; the highest soil CO_2 efflux occurred during the first 6 weeks after residue incorporation. Highest CO_2 efflux was associated with silt loam amended with either the Bt or non-Bt residues during the first 15 days of incubation compared with non-amended soil (Fig. 1). Silt loam and silty clay did not differ in CO_2 efflux except for a significant increase in silty clay amended with residues at 7 days. Efflux of CO_2 from sandy loam was about half that observed from silt loam. These results were confirmed by multiple regression analysis which showed that soil textural class ($R = 0.0114$, $P < 0.0001$) and residue component ($R = 0.0063$, $P = 0.0004$) were significant factors affecting

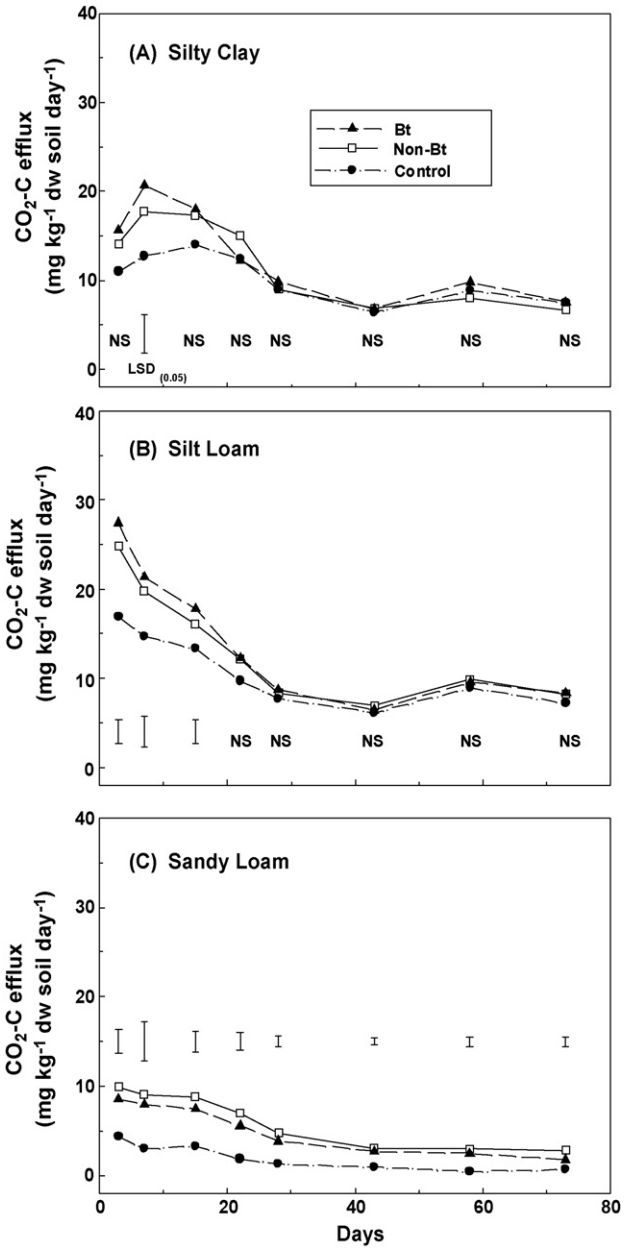

Fig. 1 – Changes in soil CO_2 efflux in response to amendment with Bt and non-Bt corn residues during 73 days of incubation in microcosms for (A) silty clay, (B) silt loam, and (C) sandy loam soils. Soil CO_2 efflux values are averaged over residue type. Vertical bars indicate $LSD_{(0.05)}$ values. Non-Bt = Non-Bt isoline, Bt = Bt corn; Control = treatment without amended residue materials.

Table 3 – Multiple regression analysis for parameters measured in the microcosm study

	Parameter							
	Soil CO_2-C efflux		Cumulative CO_2		Total plate count		AWCD[a]	
	RC[b]	P-Value	RC	P-Value	RC	P-Value	RC	P-Value
Soil	0.0114 (0.0017)[c]	<0.0001	213.4 (29.81)	<0.0001	0.2682 (0.0629)	0.0001	0.1444 (0.0223)	<0.0001
Bt	–[d]		–		−0.4122 (0.1923)	0.0369	−0.2377 (0.0965)	0.0163
Residue component	0.0063 (0.0017)	0.0004	–		–		−0.0991 (0.0316)	0.0025
Soil × Bt	–		–		0.2119 (0.0890)	0.0211	–	
Soil × Residue	–		–		–		–	
Bt × Residue	–		–		–		0.1051 (0.0447)	0.0216
Intercept	0.0274		194.0		5.628		0.4078	

$n = 3$ for soil; $n = 2$ for Bt-type corn; $n = 3$ for residue component.
[a] Average well color development.
[b] Regression coefficient.
[c] Standard error.
[d] Not statistically significant.

Table 4 – Rate constants (k^a, day^{-1}) of CO_2 evolution in the microcosms as affected by soil textural class, residue type, and residue component

Residue component	Silty clay		Silt loam		Sandy loam	
	Non-Bt	Bt	Non-Bt	Bt	Non-Bt	Bt
Leaf	0.068	0.077	0.085	0.087	0.051	0.053
Stem	0.064	0.073	0.060	0.071	0.047	0.039
Root	0.072	0.063	0.067	0.073	0.048	0.037
Control	0.051		0.059		0.028	

[a] $k = \ln(C_t - C_0)/(t - 0)$, where C_0 is the CO_2 evolution rate at day 0, C_t the CO_2 evolution rate at day 43, t is 43 days and c is the lag time. The lag time was 7 days for silty clay and 3 days for silt loam and sandy loam.

soil CO_2 evolution rates (Table 3). No significant interactions among the treatments were observed. Soil CO_2 efflux from the unamended control was significantly lower than that of the amended soils only during the first month; CO_2 efflux from soils amended with Bt and non-Bt corn residues did not differ, regardless of soil texture.

To compare differences in CO_2 evolution among residue components, kinetic rate constants (k) for each tissue were calculated using an exponential model (Table 4). During the period of most active CO_2 efflux (43 days), first-order kinetics described patterns of CO_2 evolution. The k-values of leaf components in each soil were higher than those of stem components, indicating a rapid decomposition of leaves. Soils amended with stems and roots did not differ in soil CO_2 production. Residue components positively correlated with rate of CO_2 evolved ($P = 0.0004$) (Table 3). The control soils generated low k-values as compared with their amended counterparts.

Cumulative CO_2 released after the 73-day incubation is shown in Fig. 2. The sandy loam generated the lowest cumulative CO_2 compared with that of the silt loam and silty clay. Regression analysis revealed that soil texture significantly affected cumulative CO_2 evolution ($P < 0.0001$) (Table 3). Interestingly, residue components were not significantly correlated with cumulative CO_2 production after 73 days of incubation, yet were significantly related to CO_2 efflux during the initial stage of incubation. Furthermore, total cumulative CO_2 released during the incubation was not significantly affected by residue amendments in the silt loam and silty clay. Finally, residue type (Bt and non-Bt) did not significantly affect cumulative CO_2 released, which was similar to previous research reporting that rates of soil CO_2 evolution were not affected by corn residue type (Hopkins and Gregorich, 2003).

3.4. *Culturable soil bacteria*

In the microcosm study, numbers of culturable bacteria (including actinomycetes) in silty clay and silt loam ranged

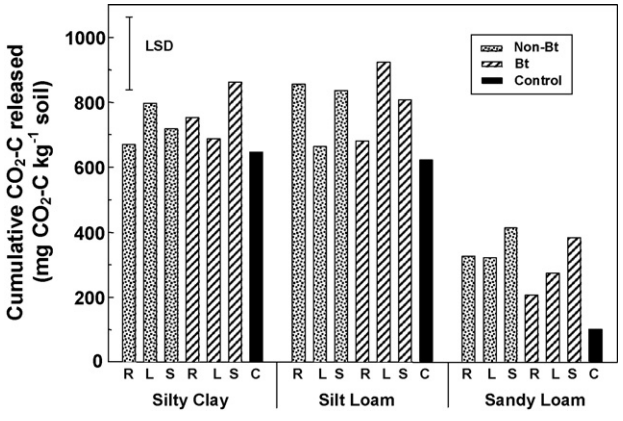

Fig. 2 – Cumulative soil CO_2 released after 73 days of incubation of silty clay, silt loam, and sandy loam soils. R = root, L = leaf, S = stem; Bt = Bt corn, non-Bt = non-Bt isoline; Control = treatment without amended residue materials.

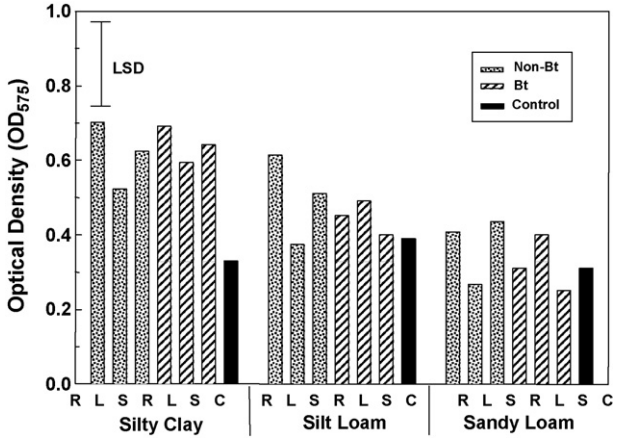

Fig. 3 – Average well color development (AWCD) in Biolog microplates after incubation at 28 °C for 72 h for silty clay, silt loam and sandy loam soils, respectively in microcosms. R = root, L = leaf, S = stem; Bt = Bt corn, non-Bt = non-Bt isoline; C = control without amended residue materials.

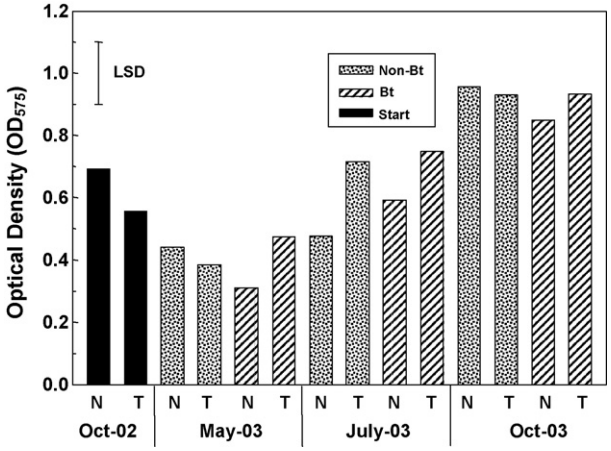

Fig. 4 – Average well color development (AWCD) in Biolog microplates after incubation at 28 °C for 72 h for a silt loam soil from the field study. Sampling times are October 2002, May, July, and October 2003. N = no-tillage, T = tillage.

from 1.0×10^6 to 5.0×10^6 CFU g^{-1} oven dry soil; the sandy loam contained lower microbial populations within the range of 3.0×10^5 to 6.3×10^5. Amendment of soils with either Bt or non-Bt corn residues had little impact on bacterial populations in silt loam and sandy loam and slightly stimulated populations in silty clay, suggesting interactions between corn residue and soil texture in which substrates or enzymes of decomposition are affected by soil particles or organic matter components.

In the field study, culturable soil bacteria were higher (6.9×10^6 to 7.4×10^7 CFU g^{-1} dry soil) compared with the incubation study. Effects of Bt residues incorporated in soils on bacterial numbers were not significantly different from those of non-Bt residues. Neither sample date nor tillage operation significantly affected culturable bacterial numbers; no interactions among soil texture, sample date, and tillage operation in the field study were detected.

3.5. *Carbon substrate utilization*

Carbon substrate utilization (AWCD) was significantly different ($P < 0.0001$) among all soils in the microcosm study (Fig. 3). The silty clay generally had higher AWCD than the silt loam and sandy loam; the sandy loam had the lowest AWCD, which coincided with low bacterial numbers and cumulative soil CO_2 efflux. Addition of corn residues increased AWCD in the silty clay compared to the unamended control, regardless of residue type or component. No significant differences in AWCD between treatments in either the silt loam or sandy loam were observed.

AWCD consistently increased throughout the growing season for all residue amendments in the field (Fig. 4). Soils sampled in October 2003 exhibited the highest AWCD. No significant differences in AWCD between Bt and non-Bt residue amendments were observed in the field study.

Principle component analysis illustrated that carbon substrate utilization patterns of bacterial communities between soils were highly significantly different ($P < 0.0001$), while they did not differ due to residue component amendment in the microcosms (Table 5). Soils amended with Bt residues significantly differed ($P = 0.0219$) from those with non-Bt residues. Similarly, a significant effect on carbon substrate utilization patterns by Bt residues was observed in the field study ($P = 0.0267$). Bacterial communities in field-collected soil significantly differed among all sampling dates based on eight PCs ($P < 0.0001$), indicating a temporal effect on

Table 5 – Comparison of microbial communities from soil in the incubation and field studies

Soil samples	# of PCs (cumulative contributed)	*F*-Value (d.f.)	*P*-Value
Microcosm			
Bt vs. Non-Bt	14 (75%)	2.15 (14–57)	0.0219
Soil texture	15 (75%)	4.97 (30–134)	<0.0001
Residue component	14 (75%)	0.91 (28–110)	0.596
Field			
Bt vs. Non-Bt	7 (76%)	2.59 (7–40)	0.0267
Sampling time	8 (76%)	11.08 (24–131)	<0.0001
Tillage vs. no-tillage	7 (76%)	0.44 (7–40)	0.871

$n = 3$ for soil texture and $n = 3$ for residue component in microcosm study with four replications. $n = 4$ for sampling time in field study with four replications.

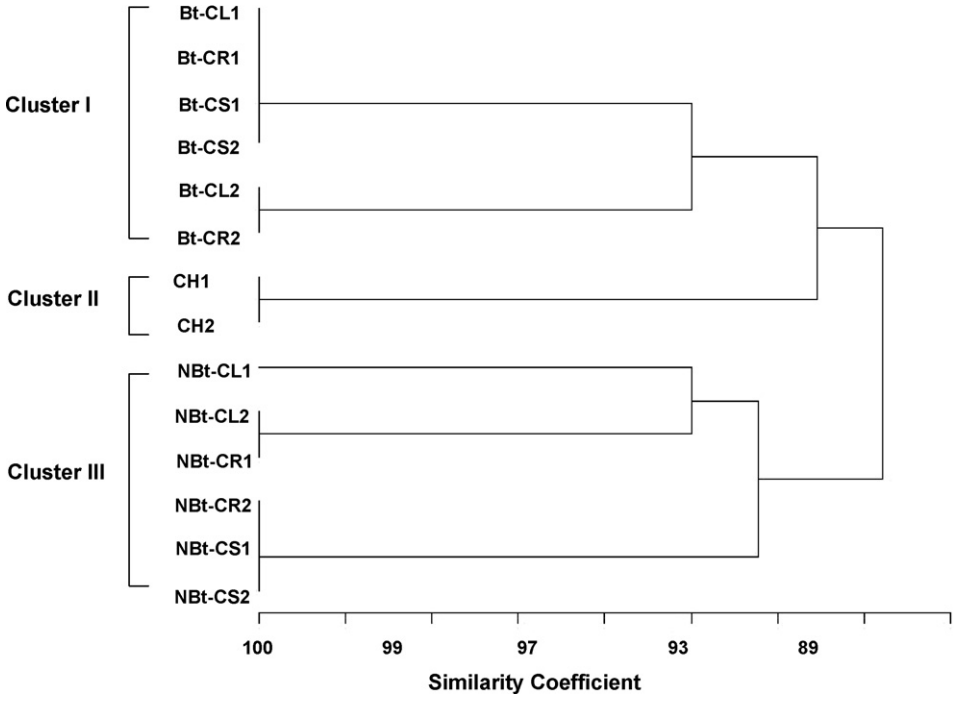

Fig. 5 – Cluster analysis of DGGE profiles of the soils using unweighted pair group with mathematical averages, UPGMA. Bt = Bt corn; NBt = non-Bt isolines; CL = leaf, CR = root, CS = stem. CH = control without residue addition.

bacterial community function. However, bacterial communities were not different tillage treatments ($P = 0.871$).

3.6. *Soil microbial communities studied by DGGE*

About 25–30 DNA bands with various intensities were detected in DGGE profiles for microbial communities in silt loam amended with either corn residue in the microcosms. Most bands were present in all treatments with minor differences detected in some samples. Cluster analyses revealed that samples formed separate groups between Bt and non-Bt residue treatments, but the difference was small with 90% similarity (Fig. 5). The controls without residue amendment grouped together in Cluster II, which shared similarity with Bt residue-amended counterparts. More than 93% similarity of DGGE patterns was noted among the soil samples amended with different plant components.

DGGE profiles of DNA extracted from field-collected soils were consistent over time, but differed in band number and intensity compared with the microcosm study, suggesting that bacterial diversity in the field environment was inherently stable. Residue type (Bt versus non-Bt) or tillage had little effect on DGGE patterns. However, a number of low-intensity bands differed across the various sample dates. It is possible that many weak bands that were not detected occurred on all sampling dates, thus distinct communities associated with the different residues may have been overlooked. However, because bands were selected based on set limits of density values, these differences were not documented. Cluster analysis revealed a 15% difference among all treatments taken at different sample dates (Fig. 6). Based on the formation of distinct groups of DGGE patterns, sampling time had a larger impact on the bacterial community structure. Samples taken in October 2002 (Fig. 6, Cluster I) were distinguishable from 2003 samples. However, the DGGE patterns from Bt and non-Bt residue treatments did not form separate groups in the dendrogram, indicating that Bt residues did not affect bacterial community structure.

4. Discussion

We addressed the potential impact of transgenic corn residue on soil microbial communities and processes, which may occur because of differences in the chemical composition, quantity, and botanical form of Bt corn residues, soil properties, temporal variation, and changes in management practices. Generally, incorporation into soil of a Bt residue, containing high lignin content and lignin/N ratio, altered functional activity (substrate metabolism) and structure of microbial communities compared with the non-Bt isoline.

The activity and diversity of soil microorganisms are often affected by the quality of vegetative residues incorporated in soil based on the extent nutrient availability in the various residues (Bending et al., 2002). Among the corn varieties grown in the field study, Merschman M-0012Bt was significantly higher in lignin content and lignin/N ratio than its non-Bt isoline (M-00110) (Mungai et al., 2005). Thus, the chemical composition of Bt corn varieties may not differ greatly from non-Bt varieties, which may partly explain contradictory results reported in other studies involving residue decomposition (Escher et al., 2000; Saxena and Stotzky, 2001b). Bt corn residue with 2× the lignin content as the non-Bt near-isoline decomposed at a significantly lower rate when incorporated in a sandy loam

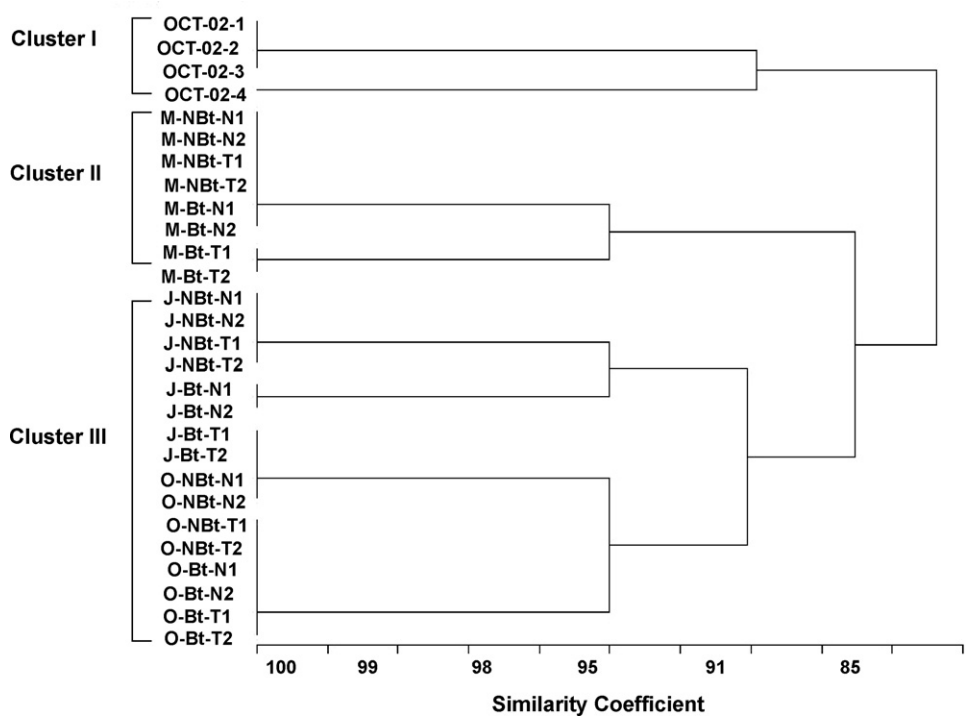

Fig. 6 – Cluster analysis of DGGE profiles of the soils from the field study using unweighted pair group with mathematical averages, UPGMA. OCT-02 = soils sampled in October 2002; M, J, and O = soils sampled in May, July and October 2003; NBt = non-Bt isolines; Bt = Bt corn; N = no-tillage, T = tillage.

(Flores et al., 2005). Similarly, we used residues of Bt corn with high lignin content and its non-Bt isoline with low lignin to study the effects of corn residue quality on soil microbial communities. Our incubation and field studies revealed that Bt residues did not affect cumulative soil CO_2 efflux, rates of soil CO_2 evolution, and DGGE patterns, however, substrate utilization patterns based on Biolog metabolic profiles were altered.

The difference in residue quality between Bt and non-Bt residue may be one of the principal reasons for the observed effects on soil microbial properties. Hopkins et al. (2001) found that material from genetically modified tobacco decomposed more rapidly than material from the wild-type plants, which was most likely the result of lower lignin content of these modified plants. Bt corn leaves with low lignin content decomposed at a higher rate compared with that of a non-Bt variety (Escher et al., 2000). Our study demonstrated that interactions among corn residue type (Bt versus non-Bt), residue component, and soil type may alter soil microbial community function. Information from this study may be useful for improving Bt corn residue management to minimize any potential undesirable agronomic and environmental effects. Because Bt corn residues may require more residence time in soil during longer decomposition, we cannot rule out the possibility that the associated toxins remaining in soil for this extended time may also affect soil microbial activity (Flores et al., 2005).

Abiotic factors, including soil nutrient status, soil pH, topsoil depth, soil water content, soil temperature, soil aeration and agronomic practices, may influence the structure and function of soil microbial communities (Marschner et al., 2001; Girvan et al., 2003; Griffiths et al., 2003; Wolf and Wagner, 2005). In the present study, soil textural differences significantly affected soil microbial composition and soil CO_2 evolution. Among the three soil types examined in this study, the sandy loam with the lowest organic C and total N had the lowest CO_2 evolution rate, microbial population, and substrate utilization activity. Previous research with transgenic and non-transgenic corn demonstrated that bacterial diversity in the corn rhizospheres were differentiated among soil textures but not between corn varieties (Gelsomino et al., 1999; Fang et al., 2005). Our observations agree with other research demonstrating that soil texture is one of most important abiotic determinants of soil microbial communities (Bossio et al., 1998; Chiarini et al., 1998; Buyer et al., 1999; Dalmastri et al., 1999; Girvan et al., 2003; Garbeva et al., 2004).

Tillage affects the population and respiration of soil microorganisms. For example, Kladivko (2001) found that most soil organisms are greater in abundance or biomass in no-till than in conventional tillage systems. Residues remaining on the soil surface after reduced and no-tillage decompose more slowly than buried residues (Schomberg et al., 1994). We hypothesized that different tillage systems may affect decomposition of Bt and non-Bt corn residues and the soil microorganisms involved in decomposition due to differences in soil disturbance and physical incorporation of residues. However, we did not detect significant effects of tillage on soil microbial populations or DGGE profiles. The impact of different tillage systems on Bt and non-Bt corn residue decomposition and the soil microbial community may require longer term studies over several consecutive growing seasons.

In summary, we used several complementary techniques (C mineralization, culturable bacteria assays, Biolog metabolic

fingerprinting, PCR-DGGE) that have been widely used to study the effects of genetically modified plants on soil microbial communities and processes (Bruinsma et al., 2003). Soil type, rather than corn residue type or component, was a dominant factor in influencing the extent of decomposition. Bt corn residues seemed to affect soil bacterial community function as measured by substrate utilization assays. The ecological importance of this observation is not clear, however, it suggests that Bt residues may affect selected activities of soil bacteria carried out by specific enzymes that cannot be detected with very general assays such as the C mineralization procedure used here. More detailed studies on Bt residues interacting with specific activities of the soil microbial community are required to confirm these effects.

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