

## Effects of tillage and nitrogen rate on decomposition of transgenic Bt and near-isogenic non-Bt maize residue

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### ABSTRACT

A great deal of research has been conducted to evaluate transgenic Bt maize (*Zea mays* L.) effect on soil organisms and residue decomposition, but the effects of management practices, such as tillage and N applications have not been fully studied. The objective of this study was to examine the decomposition rate of genetically modified Bt maize residue compared to near isogenic non-Bt maize residue under different tillage and N rates in field and laboratory studies. The study was established at the Iowa State University Field Extension Education Laboratory Research Farm near Ames, Iowa in November of 2004 through 2007. The soil type of the site was Nicollet loam (fine-loamy, mixed, mesic, Aquic Hapludolls). The field decomposition study design was a replicated complete factorial with three tillage treatments: no-tillage (NT), strip-tillage (ST) and deep tillage (DT); and two maize hybrids of a transgenic, Bt variety and another of non-BT variety; at five times intervals. The laboratory study was conducted concurrently to examine the effect of different N rates and simulated tillage treatments on Bt and non-Bt residue decomposition. The hypothesis of this study is that alterations made to the physiological traits in Bt maize residue would result in changes in residue decomposition rate and those changes would vary by N and tillage management. The findings of field decomposition study show that there were no significant differences between Bt and non-Bt decomposition rate under NT. However, in one year out of two years, buried Bt maize residue showed slower decomposition rate than non-Bt maize residue. In the incubation study, Bt maize residue that was mixed with soil had significantly less residue decomposition (11% of added residue C) than Bt that was un-mixed (13% of added residue C). Non-Bt maize residue mixed or un-mixed had on average 13% of residue decomposed after 80 days. Additions of N significantly lowered decomposition rates, but did not result in significant differences between Bt and non-Bt maize residue decomposition. The relevance and application of these findings are critical in providing practical information that Bt corn residue did not significantly differ in majority of time from non-Bt residue with different tillage systems in field conditions. Also, the addition of N for the purpose of increasing residue decomposition is not warranted in this study. These findings can have value in promoting conservation practices and keeping residue on soil surface rather than incorporating it with tillage.

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### 1. Introduction

Transgenic maize that expresses insecticidal proteins derived from the bacterium *Bacillus thuringiensis* (Bt) have been used for control of many economically important lepidopteran, coleopteran, and dipteran pests (Schnepf et al., 1998). Since 1996, Cry1Ab protein expressing Bt maize has been commercially available for control of the European maize borer, *Ostrinia nubilalis*, one of the most economical damaging pests of maize in the U.S. Maize Belt (James, 2002). The Cry1Ab protein expression in maize hybrid has

proven to be an effective management option that also lessens the use of pesticide applications (Mendelsohn et al., 2003), and reduces the potential adverse effects of these compounds on the environment and human health.

Questions have been raised on the inadvertent effects of these Bt proteins on other unintended soil organisms and decomposition of Bt maize residue. Laboratory and in situ studies in general have shown that neither populations nor functions of microorganisms and micro fauna are altered in the presences of Bt proteins (Saxena and Stotzky, 2001a). However, findings on decomposition rates of Bt maize residue compared to near-isogenic non-Bt hybrids are inconclusive. Under laboratory conditions, Stotzky (2000) and Flores et al. (2005) have reported lower decomposition rates in Bt crop residue compared to non-Bt. Differences in plant composition between Bt and non-Bt hybrids have in part been attributed to the potential changes in the decomposition rates of residue and not the

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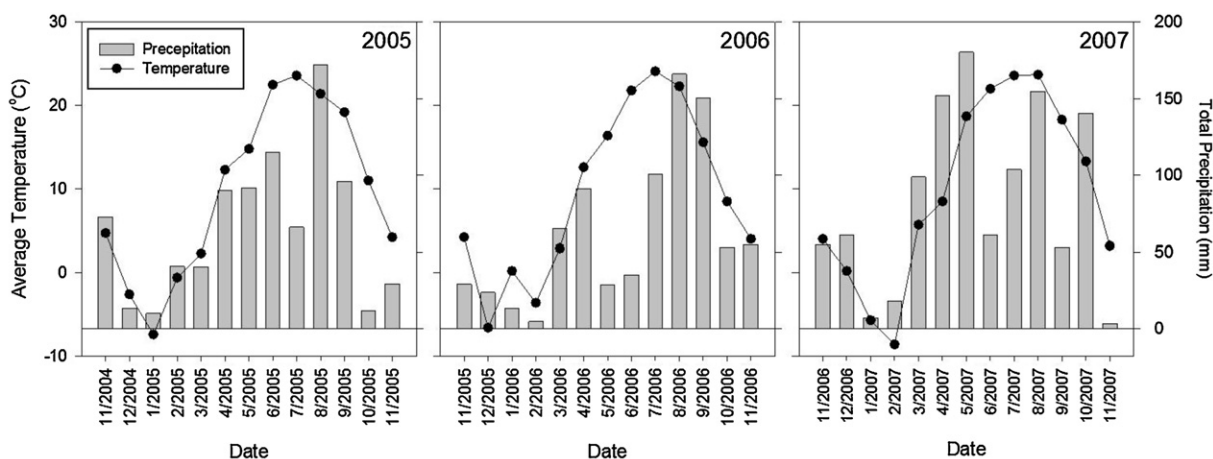


Fig. 1. Monthly average temperature and precipitation from 2004 through 2007 at the Iowa State University Field Extension Education Laboratory Research Farm.

inhibition of soil microbial activities. Saxena and Stotzky (2001b) reported that Bt hybrids can have higher lignin content compared with near-isogenic non-Bt hybrids. Plant tissues that contain higher lignin content have been shown to be more resistant to microbial decomposition than cellulose, hemi-cellulose, and starch compounds (Heal et al., 1997). Additionally, differences in carbon and lignin content lead to changes in carbon to nitrogen ratio (C:N) and lignin to N ratio (L:N), factors that heavily affect decomposition rates in plant tissues (Heal et al., 1997). However, studies have also shown no significant differences or contrary results of plant composition between Bt and near-isogenic non-Bt maize hybrids (Escher et al., 2000). The use of laboratory conditions to predict actual decomposition rates in the field are not always accurate. Reasons include changes in soil physical, biological, and chemical properties during sample preparation and storage, and the static conditions of the laboratory study (constant soil temperature and water content) versus dynamic conditions of a field study (wetting and drying and seasonal cycles).

There are limited field studies that compare decomposition rates of Bt hybrids with near-isogenic non-Bt hybrids under typical agriculture conditions. In general, these studies have shown no adverse effects in decomposition rate between crop residues that express the Bt protein with those that do not (Lachnicht et al., 2004; Cortet et al., 2006; Devare et al., 2007; Lehman et al., 2008; Daudu et al., 2009). However, most of these field studies have only looked at differences in climatic and soil textures. Different field management practices such as tillage systems, can leave crop residues on the soil surface as in no-tillage, or are incorporated into the soil exposed to sub-surface conditions as in conventional tillage system need to be examined. These differences in management can contribute differently to the rate of residue decomposition. Therefore, we hypothesized that alterations made to the physiological traits in Bt maize residue would result in changes in residue decomposition rate, although those changes would vary by N and tillage management. The objective of this study was to examine the decomposition rate of genetically modified Bt maize residue compared to its near isogenic non-Bt maize residue under different tillage systems and N application rates in field and laboratory incubation studies.

## 2. Materials and methods

### 2.1. Site description and field experiment design

This study was established at the Iowa State University Field Extension Education Laboratory Research Farm near Ames, Iowa

(42°39'N lat; 95°47'W long) in November of 2004 through 2007. The experiment was conducted on a single soil type, Nicollet loam (fine-loamy, mixed, mesic, Aquic Hapludolls) (Soil Survey Staff-USDA-NRCS, 1999). The topsoil (0–15 cm) had a pH of 5.8 ( $H_2O$ ); 241  $g\ kg^{-1}$  clay; 372  $g\ kg^{-1}$  silt; 435  $g\ kg^{-1}$  total carbon (C); 28  $g\ kg^{-1}$  total nitrogen (N); 0.02  $mg\ kg^{-1}$   $NH_4-N$ ; and 4.5  $mg\ kg^{-1}$   $NO_3-N$ . The site was in conventional tillage (chisel plow) and maize (*Zea mays* L.)-soybean [*Glycine max* (L.) Merr.] rotation. Average monthly temperature and total precipitation during the study is shown in Fig. 1. The mean daily temperature and annual rainfall for 2005, 2006, and 2007 were 10.1 °C and 819 mm, 10.0 °C and 750 mm, and 10.4 °C and 1084 mm, respectively.

The field residue decomposition study was conducted with maize residue that was placed in nylon bags 20 cm × 20 cm in a complete factorial with three treatment factors; tillage, maize hybrid, and time of residue bag removal with three replications. The three tillage systems were: no-tillage (NT), strip-tillage (ST), and deep tillage (DT). No-tillage was defined as no pre-plant tillage, where a planter with a single coulter was used to cut through crop residue and loosen soil. The only soil disturbance associated with NT was during planting and fertilizer applications. Deep tillage was done in the fall with a Yetter 5 shank in-line ripper. The shanks were set on 75 cm spacing to a depth of 38–46 cm. Strip-tillage was done with a five row Yetter Maverick style strip-tillage unit to a depth 19 cm deep. It was equipped with straight coulters in front of each knife and disk covers behind the knife to close up the trench. Maize seeds are of one transgenic, Bt hybrid and near-isogenic non-Bt hybrid (non-Bt) (Golden Harvest H-8345 (non-Bt) and H-8445 (Bt)). These were iso-lines identical except for the Bt trait. Maize residue from both hybrids left after harvest was used in the following year of soybean for the decomposition study over five time intervals (0 months, 3 months, 6 months, 9 months and 12 months) for each tillage system. The plots dimensions for maize crop and following soybean crop were 60 m by 270 m long at row width of 75 cm.

### 2.2. Maize residue field decomposition setting

Maize residue (leaves and stems) of Bt and non-Bt was collected shortly after every harvest from each maize hybrid and tillage treatments. Three hundred grams of maize residue was weighed and placed into 20 cm × 20 cm residue bags of 2 mm nylon mesh after residue was dried at 60 °C for a week before placed in bags. Residue bags were placed in the field in each tillage system during the month of November after maize harvest and left for 12 months

under soybean crop the following year. The soybean crop was planted in mid-May of every year in the same plots (60 m wide and 270 m long) with 75 cm row width. The residue bags were placed in the center of each plot with four guard rows between treatments. The NT residue bags were pinned to the soil surface with landscape metal stakes. The ST residue bags were partially buried up to 10 cm deep and DT residue bags were buried 30 cm below the soil surface. One set of residue bags that represent time zero was kept as a baseline for total C and N and composition analysis. Residue bags were collected from the field plots of each tillage system at 3 months interval for 12 months since the start of the experiment. After the removal of residue bags, maize residue was hand-sieved to remove soil and then dried at 60 °C for a week and weighed to determine weight loss. Maize residue samples were then ground and analyzed for total C, total N, and plant composition. The concentrations of total C and total N of maize residue was determined by dry combustion with CHN analyzer (LECO, St. Joseph, MI). Hemicellulose, cellulose, soluble cellulose, and lignin were determined by sequential detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) procedures with an ANKOM 200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY) as corrected for ash (Undersander et al., 1993).

### 2.3. Laboratory incubation experiment design

A laboratory incubation study was conducted simultaneously to examine the effect of different N rates on both Bt and non-Bt residue decomposition. Soil used in the incubation study was collected from the same field study from the top 15 cm depth. A static incubation-titrimetric procedure (Zibilske, 1994) was used in this experiment to measure C loss from residue as CO<sub>2</sub>. The soil was sieved (2-mm diameter), and allowed to air dry. One hundred grams of air dry soil sample was weighed and placed into a 0.9-L glass jar (wide mouth). Water was added to the soil to achieve 60% water filled pore space in addition to periodic water additions to maintain soil water content. Jars were then treated with a complete randomized design of 4 g maize residue (Bt and non-Bt) that was ground to pass a 2 mm screen and three NH<sub>4</sub>NO<sub>3</sub>-N rates equivalent to 80, 160, 320 kg ha<sup>-1</sup> with three replications. Control soils without straw and fertilizer N were included as well, and empty incubation jars were used as controls for CO<sub>2</sub> absorbed from the atmosphere during the incubation procedure and measurements. A 20 mL scintillation vial containing 5 mL of one N NaOH as a base trap to capture evolved CO<sub>2</sub> was placed in each jar. All containers were placed in a dark incubation room at 30 °C constant temperature. The amount of CO<sub>2</sub>-C evolved was determined by titration. Five mL of 2 N BaCl<sub>2</sub> and 2–3 drops of phenolphthalein were added to the base traps. The trap solution was then titrated against one N HCl using digital micro buret until the indicator showed a neutral pH. After titration, a new base trap was prepared and placed in each jar. Titrations were performed on days 1, 3, 5, 7, 9, 11, 18, 25, 39, 53, and 82 after placement in incubation room. Amount of residue C mineralized was calculated by subtracting amounts of CO<sub>2</sub>-C evolved from treatments without residue from those with residue of each N treatment. Residue remaining was estimated by difference between amounts of residue C mineralized and amount of residue C of the original mass used in the experiment. First-order rate constants (*k*) describing

initial rates of residue decomposition were calculated by using the following model from Murwira et al. (1990).

$$C_t = C_0(1 - e^{-kt})$$

where *C<sub>t</sub>* is carbon content at time *t* (day), *C<sub>0</sub>* is initial C content, *k* is first-order rate constant, and *t* is time (day). Using decay coefficient *k* values, time required for 50, 75, 90, and 99% residue C mineralization was estimated by:

$$t_{0.50} = \ln \frac{2}{k}$$

$$t_{0.75} = \ln \frac{4}{k}$$

$$t_{0.90} = \ln \frac{10}{k}$$

$$t_{0.99} = \ln \frac{100}{k}$$

### 2.4. Statistical analysis

First-order rate constants (*k*) were estimated using nonlinear model (nls) parameter estimation in R software (version 2.13.2). Least square means of weight loss in the field, plant composition contents, and percentage of C mineralized was performed by general linear procedure (GLM) (SAS Institute, 2002). Statistical significance was evaluated at *P* ≤ 0.05.

## 3. Results

### 3.1. Tillage effect on Bt and non-Bt maize residue decomposition in the field

Analysis of composition of fresh Bt and non-Bt maize residue (leaves and stems) after harvest show no significant difference between the two types of residue for soluble cellulose, cellulose, hemicellulose, lignin, C, and N fractions (Table 1). In general, these residue compositions content decreased over the 12 month period after harvest during the field study decomposition, although differences between Bt and non-Bt maize residue were not significantly different (data not shown).

In 2005, around 11–22% of the maize residue mass was decomposed over winter when the ground was predominately frozen (Fig. 2). After 6 months in the field, soil had thawed and soil temperature increased (March through May), maize residue that remained on the soil surface ranged from 45 to 60%. After 9 months in the field, maize residue that remained ranged from 19 to 32%. There were no further significant decreases in maize residue mass observed after 9 months. Additionally, there were no significant differences in percentage of maize residue that remained among the three tillage systems (DT, ST and NT) and Bt and non-Bt maize hybrids residues in any time during the year.

In 2006, 12–26% of the maize residue mass was decomposed over winter from the newly collected maize residue after harvest in November 2005 (Fig. 2), which is similar to 2005 results. There were no significant differences in the percent of residue that remained among the three tillage systems of Bt and non-Bt maize hybrids decomposition. After 9 months in the field, maize residue

**Table 1**  
Initial plant composition of Bt and non-Bt maize residue and total C, total N, and C:N ratio. Mean ± one standard deviation.

Hybrid	Total C (g kg <sup>-1</sup> )	Total N (g kg <sup>-1</sup> )	Soluble cellulose (%)	Hemicellulose (%)	Cellulose (%)	Lignin (%)	C:N
Bt	44.2 ± 0.17	0.97 ± 0.02	20.1 ± 0.21	32.3 ± 0.48	39.4 ± 0.26	3.4 ± 0.11	47.0
Non-Bt	42.1 ± 0.16	0.98 ± 0.02	20.2 ± 1.46	33.1 ± 1.23	38.7 ± 0.97	3.1 ± 0.12	47.1

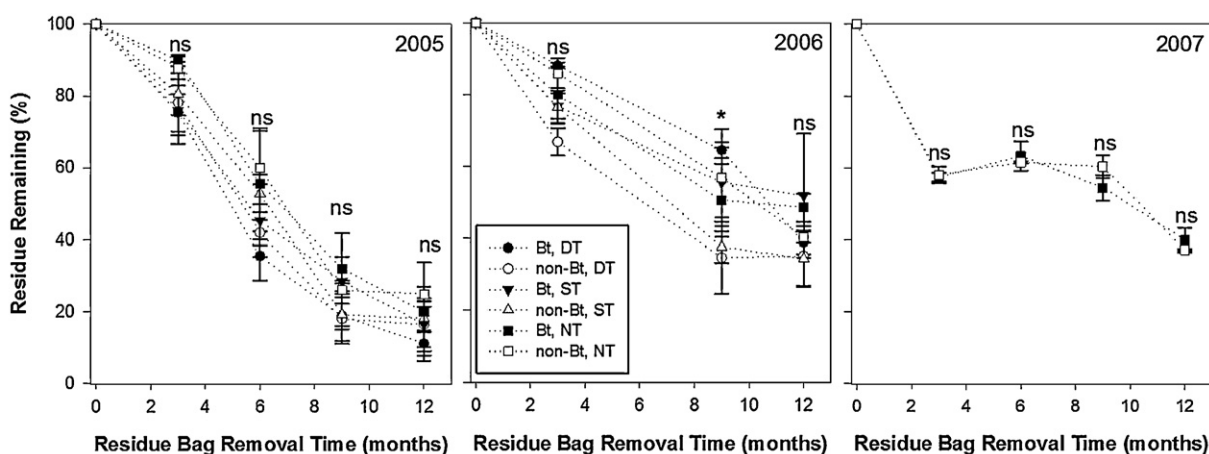


Fig. 2. Percent of Bt and non-Bt maize residue remaining at different residue removal times under different tillage managements in 2005, 2006, and 2007. Error bars represent the standard error of treatment means.

that remained ranged from 38 to 57%. There was a significant tillage  $\times$  hybrid interaction effect on the percentage of residue that remained. Also, after 9 months the Bt maize hybrid residue that was either fully (DT) or partly (ST) buried had significantly greater residue percentage that remained compared to non-buried Bt maize and buried or non-buried non-Bt residues. However, these differences were not observed after 12 months in the field where 34–49% of the maize residue still remained.

In 2007, differences between Bt and non-Bt maize residue decomposition was only evaluated in NT system (Fig. 2). The largest decreases in maize residue mass occurred after the first three months after harvest, which resulted in an average loss of 43% over the winter. This can be attributed to a warmer month in March compared to 2005 and 2006 (Fig. 1). The percentage of maize residue that remained on the soil surface stayed constant between the 3 and 9 months periods until further decreases were observed between 9 and 12 months under NT (18%). There were no significant differences between Bt and non-Bt maize hybrids. Changes in total C and total N contents in maize residue over time under NT management were not significantly different between Bt and non-Bt maize (Fig. 3). In general, decreases in total C content were observed while total N content remained relatively unchanged in the maize residue. Consequently, C/N ratios decreased from 47 to 38 after 12 months of decomposition in the field.

### 3.2. Effects of nitrogen application on Bt and non-Bt maize residue decomposition in a laboratory incubation study

An initial mineralization flush occurred during the first 5 days of total  $\text{CO}_2\text{-C}$  evolved from maize residue added and C released from the soil (Fig. 4). The C flux was much higher from soils treated with maize residue than those without residue (controls) after the first 55 days. Net cumulative C evolved (C treated soils minus controls) showed a significant residue type  $\times$  mixing  $\times$  time interactions effects when differences between Bt and non-Bt maize residue occurred (Fig. 5). The Bt maize residue that was mixed with soil had significantly less C mineralization ( $189 \text{ mg kg}^{-1}$ ) (11% of added residue C) than Bt that was un-mixed, while non-Bt maize residue mixed or un-mixed had on average  $212 \text{ mg kg}^{-1}$  or 13% of added residue C mineralization after 80 days across all different N rates. The additions of N did not result in differences in Bt and non-Bt maize residue C mineralization or decomposition, but it did significantly lower C mineralization overall (Fig. 6). Carbon mineralization was the greatest when no N was added at

$435 \text{ mg kg}^{-1}$  or 31% of added residue C compared to treatments of different N rate after 80 days, where the additions of N rate equivalent to 80, 160, and  $320 \text{ kg N ha}^{-1}$  resulted in 164 (10%), 128 (7%), and  $96 (5\%) \text{ mg kg}^{-1}$  C mineralization, respectively, from maize residue.

## 4. Discussion

### 4.1. Effect of tillage on Bt and non-Bt maize residue decomposition in the field

There were no significant differences in plant composition between Bt and non-Bt maize residue in this study and it is in agreement with other studies (Jung and Scheaffer, 2004; Mungai et al., 2005). However, other studies have reported greater lignin content and C/N ratios in Bt maize residue than non-Bt (Folmer et al., 2002; Poerschmann et al., 2005; Saxena and Stotzky, 2001a; Stotzky, 2004), while other studies show less lignin content in Bt maize residue (Escher et al., 2000). These discrepancies are in part may be due to how Bt and non-Bt hybrids were developed, which vary by seed company.

The decomposition rate of both hybrid types was examined through a simple first-order kinetic model,  $C_t = C_0(1 - e^{-kt})$  (Murwira et al., 1990), which was used to describe C mineralization during the initial stages of maize residue decomposition in the field study (Table 2). In 2005, Bt and non-Bt maize had similar  $k$  rates of 0.0045 for DT and ST. The half-life of the easily decomposable maize residue to mineralize was 152 days. However, under no-tillage, it took an additional 36 days to achieve 50% of Bt and non-Bt maize residue to be mineralized. In 2006, differences in  $k$  rates were observed for Bt and non-Bt maize residue. When maize residue was fully or partly buried as in DT and ST, lower C mineralization rates were observed for Bt compared to non-Bt. Under DT and ST, Bt residue  $k$  rates were 44% and 31% lower compared to non-Bt, respectively. On the other hand, residue left on the soil surface as in NT, C mineralization was lower compared to DT and ST ( $0.0022k$ ), although there were no significant differences between Bt and non-Bt residue mineralization. It has been documented that the presence of Bt proteins in the soils due to residue decomposition do not alter populations or functions of microorganisms and micro fauna (Saxena and Stotzky, 2001a). Other studies however, did show that in clay soils (>40% clay), where Bt maize was grown, the presence of eukaryotic microorganisms were decreased, although it was not clear which groups of eukaryotes (bacteria or fungi) were affected or how biologically

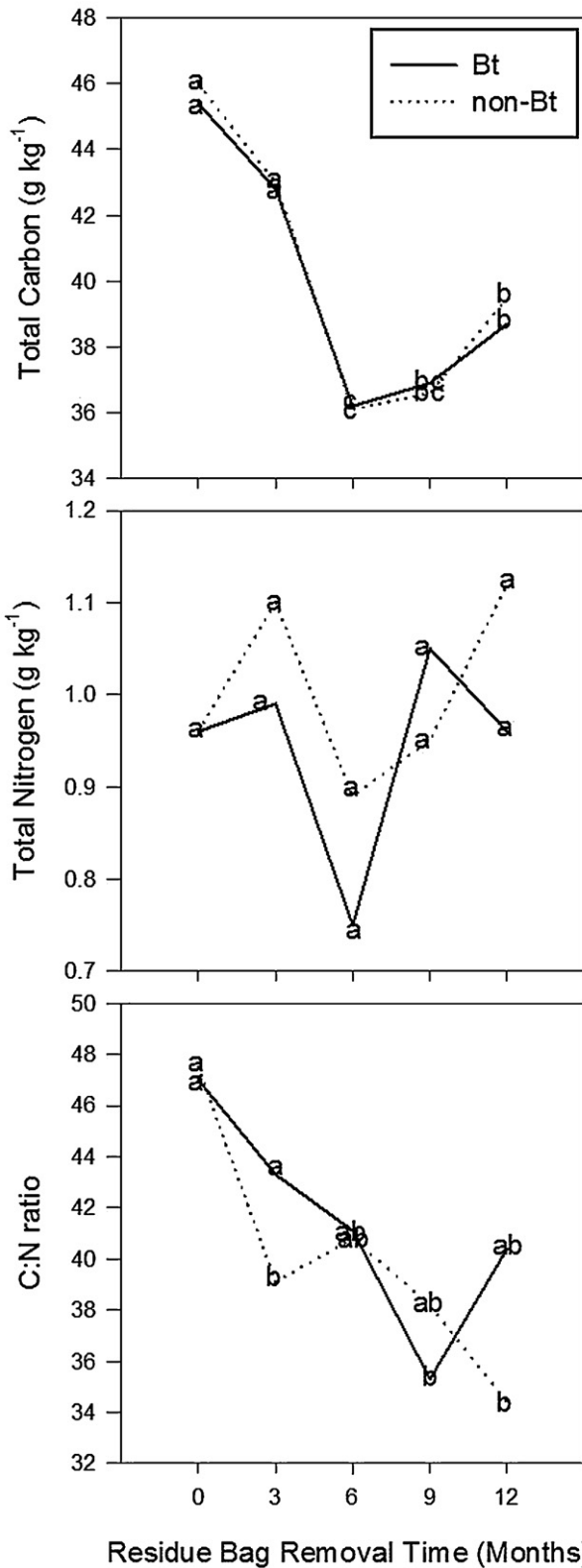


Fig. 3. Total C and N concentrations and C:N ratios of Bt and non-Bt maize residue at different residue removal times. Values with the same letter are not significantly different at the 0.05 probability level using least significant difference.

important is such trend. Further details of the microbial community composition (bacteria and fungi) and how it is affected by the tillage and N practices, soil type, and climate is required to understand the effect of the Bt protein on residue decomposition. Tillage systems effect on microbial community were examined by

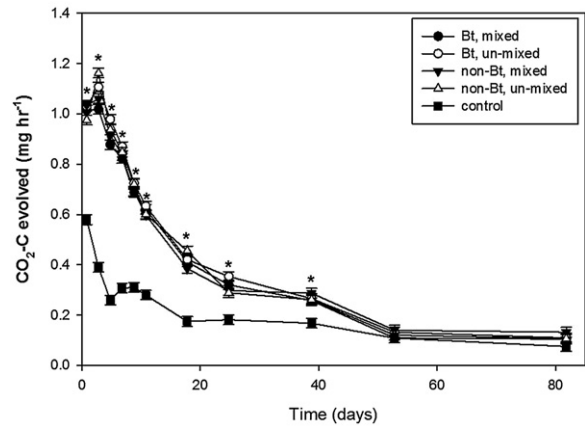


Fig. 4. Rate of CO<sub>2</sub>-C released from control (no residue added) and Bt and non-Bt maize residue mixed or un-mixed with soil. Asterisk represents CO<sub>2</sub>-C released rates from treatments that were significantly different from control at the corresponding days at the 0.05 probability level using least significant difference.

Beare et al. (1993), as well, where they found that under NT management, fungal communities associated with sorghum residues left on the soil surface, differed from those soil fungal communities. However, this was not observed when residues were buried under CT, where there was a lack of specialized fungal communities and they were significantly different from those in NT soils. Although differences in microbial communities between CT and NT were not measured in our study, this may aid in explaining why buried Bt residue had lower *k* rates than Bt residue in NT in 2006 (Table 2). Additionally, Saxena et al. (2002) showed that the Bt protein is retained longer in higher clay soils, allowing for more exposure to microbes in the rhizosphere. In one study in South Eastern United States, where effects of tillage on Bt and non-Bt cotton residue were examined, there were no differences in decomposition rates between CT and NT observed (Lachnicht et al., 2004). In that study, residue decomposition was only measured during the winter months in well-drained sandy clay loam floodplain soil with 21% clay content (fine loamy siliceous thermic Rhodic Hapludult) and with more readily decomposable plant material compared to maize leaves and stalks used in this study. Other field residue bag studies have also shown no differences between Bt and non-Bt maize residue decomposition when buried such as in Lehman et al. (2008) in Barnes clay loam soils in North Central United States (fine-loamy, mixed, superactive, frigid Calcic

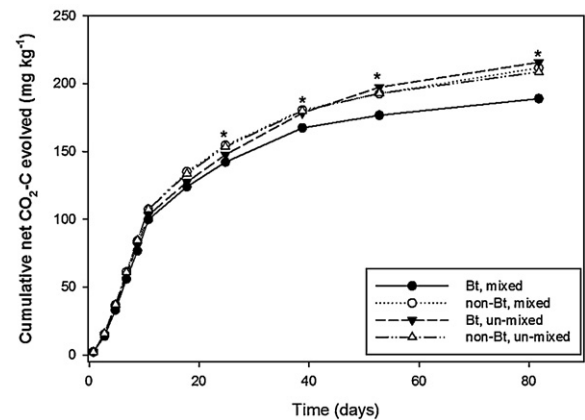
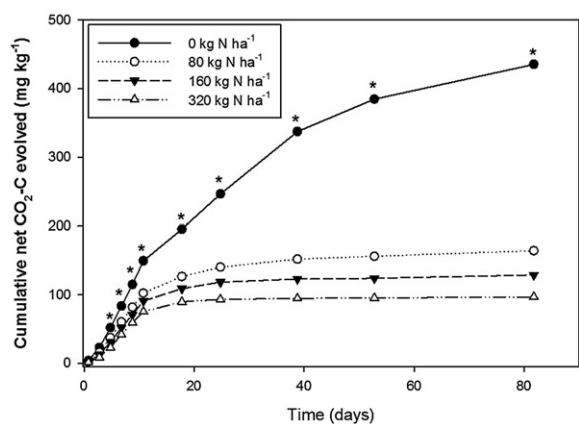


Fig. 5. Cumulative CO<sub>2</sub>-C evolved, at various times during the incubation of mixed and un-mixed maize residues (Bt and non-Bt) with soil averaged across all N rates. Asterisk represents days when significant differences of cumulative CO<sub>2</sub>-C evolved occurred between treatments at the 0.05 probability level using least significant difference.



**Fig. 6.** Cumulative  $\text{CO}_2\text{-C}$  evolved, at various times during the incubation of maize residue under different N rates averaged across residue types and mixing treatments. Asterisk represents days when significant differences of cumulative  $\text{CO}_2\text{-C}$  evolved between treatments were observed at the 0.05 probability level using least significant difference.

Hapluudoll, 28% clay); Daudu et al. (2009) in a Eutric Cambisol in South Africa (17.6% clay). Maize residue left on the soil surface in general tended to have lower decomposition compared to buried residue treatments of ST and DT in 2005 and 2006. This can be attributed to greater residue–soil contact, greater subsurface soils water content, and potential exposure to greater decomposing organisms densities compared to surface conditions (Beare et al., 1992). In 2007, there were no significant differences between Bt and non-Bt  $k$  rates (0.0027) in NT. These findings highlight the importance of our study in addressing the role of tillage system in residue decomposition, especially with Bt residue where speculations of slow Bt residue decomposition are used as justification for tillage practices (Table 3).

#### 4.2. Effects of nitrogen application on Bt and non-Bt maize residue decomposition in a laboratory incubation study

The same first-order kinetic model ( $C_t = C_0(1 - e^{-kt})$ ) used in the field residue bag study was used for the incubated soils and residue under different (mixed and un-mixed) treatments. Assuming constant decay rates for specific treatments, time periods required

**Table 2**

First order  $C_t = C_0(1 - e^{-kt})$  decay model describes C mineralization of easily decomposable Bt and non-Bt maize residue in field residue bag study under deep-tillage (DT), strip-tillage (ST) and no-tillage (NT) by year.

Hybrid	Tillage	$k^*$ ( $\text{day}^{-1}$ )	$R^2$	P-value	$t_{0.5}$ (days)
<b>2005</b>					
Bt	DT	0.0047 a	0.92	<0.0001	147
Non-Bt	DT	0.0048 a	0.91	<0.0001	144
Bt	ST	0.0044 a	0.86	<0.0001	157
Non-Bt	ST	0.0043 a	0.89	<0.0001	161
Bt	NT	0.0032 b	0.93	<0.0001	216
Non-Bt	NT	0.0032 b	0.93	0.0028	216
<b>2006</b>					
Bt	DT	0.0020 b	0.93	0.0015	346
Non-Bt	DT	0.0037 a	0.92	0.0006	187
Bt	ST	0.0022 b	0.90	0.0006	315
Non-Bt	ST	0.0032 a	0.88	<0.0001	216
Bt	NT	0.0022 b	0.95	0.0006	315
Non-Bt	NT	0.0022 b	0.94	<0.0001	315
<b>2007</b>					
Bt	NT	0.0027 a	0.95	<0.0001	256
Non-Bt	NT	0.0027 a	0.94	<0.0001	256

\* Mean  $k$  values within each year with the same letter are not significantly at  $P \leq 0.05$ .

for 50, 75, 90 and 99% C mineralization was calculated. Decomposition rate constants ( $k$ ) were relatively the same in Bt and non-Bt maize residue when residue was un-mixed with soil. Half-lives ( $t_{0.5}$ ) of the easily decomposable maize residue to occur in non-Bt and Bt un-mixed with soil were estimated to be 7 and 22 days for 90% ( $t_{0.9}$ ). However, Bt maize residue that was mixed with soil had a significantly lower decomposition rate constant ( $k$ ), resulting in additional 5 days for 90% of the easily decomposable maize residue to occur. It is uncertain why Bt maize residue when incorporated into the soil had lower decomposition rates compared to non-Bt, but there were no differences when both residue types were left on the soil surface. This was also observed in the field residue study in 2006, but not in 2005. Potential reasons for such outcome can be due to the role of residue–soil contact, subsurface soils water content, soil temperature differences, and differences in the densities of decomposing organisms in affecting buried Bt residue compared to non-Bt (Beare et al., 1992).

The additions of N significantly decreased maize residue C mineralization (Table 4), but did not significantly affect differences

**Table 3**

First order  $C_t = C_0(1 - e^{-kt})$  decay model describes C mineralization of easily decomposable maize residue added to the soil as mixed (+) or not (–) with soil across different nitrogen rates. Using decay coefficient ( $k$ ) values and assuming constant decay rates for specific treatments, half-lives ( $t_{0.5}$ ) and days required for 75% ( $t_{0.75}$ ), 90% ( $t_{0.90}$ ) and 99% ( $t_{0.99}$ ) residue C mineralization were estimated.

Hybrid	Mixing (–/+)	$k^*$ ( $\text{day}^{-1}$ )	$R^2$	P-value	$t_{0.5}$ (day)	$t_{0.75}$ (day)	$t_{0.9}$ (day)	$t_{0.99}$ (day)
Bt	–	0.1043 a	0.97	<0.0001	7	13	22	44
Non-Bt	–	0.1071 a	0.96	<0.0001	6	13	22	43
Bt	+	0.0863 b	0.98	<0.0001	8	16	27	53
Non-Bt	+	0.1027 a	0.96	<0.0001	7	13	22	45

\*  $k$  values with the same letter are not significantly different at the 0.05 probability level using least significant difference.

**Table 4**

First order  $C_t = C_0(1 - e^{-kt})$  decay model describes C mineralization of easily decomposable maize residue added to the soil by nitrogen rate across maize hybrids and mixing treatments effects. Using decay coefficient ( $k$ ) values and assuming constant decay rates for specific treatments, half-lives ( $t_{0.5}$ ), 75% ( $t_{0.75}$ ), 90% ( $t_{0.90}$ ), and 99% mineralization percentage and number of days required for each residue C mineralization rate.

N addition ( $\text{kg N ha}^{-1}$ )	$k^*$ ( $\text{day}^{-1}$ )	$R^2$	P-value	$t_{0.5}$ (day)	$t_{0.75}$ (day)	$t_{0.9}$ (day)	$t_{0.99}$ (day)
0	0.1873 a	0.88	<0.0001	4	7	12	25
80	0.0992 b	0.98	<0.0001	7	14	23	46
160	0.0695 c	0.96	<0.0001	10	20	33	66
320	0.0369 d	0.95	<0.0001	19	38	62	125

\*  $k$  values with the same letter are not significantly different at the 0.05 probability level using least significant difference.

between Bt and non-Bt maize residue decomposition rates. The half-lives were estimated on average of 4 days, and for 90% of the easily decomposable maize residue, it took 12 days to occur for Bt and non-Bt. The addition of N equivalent to 80 N kg ha<sup>-1</sup> to incubated soils, lead to a significantly lower *k* values resulting in doubling number of days for the easily decomposable maize residue to reach their half-lives and 90% C mineralization of 7 and 24 days, respectively. Further increase of N rate to 160 kg ha<sup>-1</sup> resulted in decreasing *k* values at a relatively linear rate of 0.03. There is no consensus on how N affects C mineralization, with some studies showing a suppressive effect of N on C mineralization and others showing a stimulatory effect (Fog, 1988; Green et al., 1995). One reason that was given on why N additions suppress C mineralization and CO<sub>2</sub> evolution is that mineral N inputs may lead to increased decomposer efficiencies when N is continuously supplied (Agren et al., 2001). That means an enhancement in residue decomposition integration into soil organic matter instead of CO<sub>2</sub> emission. These increases in decomposing efficiencies are due to changes in decomposing communities that have a greater N requirement (Agren et al., 2001). Further evidence are reported by Moran et al. (2005) study that shows greater residue-C was transformed into humin-C with mineral-N input. In this incubation study, maize residue treatments with N application appeared to have decomposed less, using CO<sub>2</sub> evolution as an indicator of decomposition. This might be attributed to the N additions effect in acidifying the soil and lowering pH further, which had an initial pH of 5.8–4.6. Additions of N fertilizers such as NH<sub>4</sub>NO<sub>3</sub> have been shown to reduce soil pH (Schwab et al., 1989), which have been linked to reducing microbial activities (Rousk et al., 2009). Furthermore, Green et al. (1995) showed that additions of N can have suppressive effects on C mineralization from SOM, but it can be stimulatory for maize residue that is added. In their study, under lower rates of 2 g residue per kg of soil, addition of N had an overall suppressive effect on CO<sub>2</sub> evolution and C mineralization (Table 4) as in this study (Fig. 6 and Table 4). However, under higher rates of added maize residue (>2 g residue per kg of soil), N addition had a greater stimulatory effect on CO<sub>2</sub> evolution and C mineralization.

If Bt maize residues were to decay differently, this would have significant ramifications on overall soil quality. Longer persistent plant material in the soil or on the surface tend to increase accumulations of soil carbon (Al-Kaisi and Yin, 2005), soil aggregation (Six et al., 2000), nutrient cycling (Brye and Pirani, 2005), microbial biomass and diversity (Grote and Al-Kaisi, 2007; Allison et al., 2005) and reduce water and wind erosion (Karlen et al., 1994). Additionally, the importance of crop residue left after harvest is largely due to its influence on soil water content and soil temperature, which are highly dependent on soil type, soil drainage, climate conditions, and tillage system (Al-Kaisi et al., 2005). Hence, a soil in good physical and biological health entails an environment that favors a healthy microbial community that breaks down crop residue to improve nutrient cycling processes and soil structure. Harmful effects from remaining maize residue in the soil can also occur. The survival of plant pathogens like *Fusarium* (Naef and Defago, 2006) from previous year's crop residue can cause more disease problems.

## 5. Conclusions

The field findings from this study show that under NT systems, there were no significant differences between Bt and non-Bt maize residue decomposition rates. However, under DT and ST systems in one out two years, Bt maize residue when buried decomposed at lower rate than buried non-Bt, although similar to that of NT. Soil incubation findings also showed that Bt maize residue decomposed at a lower rate than non-Bt, but only when mixed into the soil. Additionally, N application did not have a

significant effect on differences between Bt and non-Bt maize residue decomposition rates, but did result in lower decomposition rates as N rate increased. These findings answer some of the effects of both tillage and N management on Bt and non-Bt residue decomposition. The relevance and application of these findings are critical in providing practical information that Bt residue decomposition did not significantly differ in majority of time from non-Bt residue with different tillage systems. Also, the addition of N for the purpose of increasing residue decomposition is not warranted. These findings can have value in promoting conservation practices and keeping residue on soil surface rather than incorporating it with tillage.

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