BT VS. NON-BT CORN (*ZEA MAYS* L.) HYBRIDS:

EFFECT ON DEGRADATION OF CORN STOVER IN SOIL

A Thesis

by

HERMINIA TERESA SALVATORE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2009

Major Subject: Soil Science

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Approved by:

Co-Chairs of Committee,	Frank M. Hons David A. Zuberer
Committee Member,	Donald A. Sweeney
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ABSTRACT

Bt vs. Non-Bt Corn (*Zea mays* L.) Hybrids:

Effect on Degradation of Corn Stover in Soil. (May 2009)

Herminia Teresa Salvatore, B.S., Universidad Tecnologica Nacional, Argentina

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A billion tons per year of genetically modified corn residues are soil incorporated having both direct and indirect effects on the belowground environment, soil carbon (C) sequestration, and nutrient cycling. If Bt genetic modification has non-target effects on corn stover structural/non-structural carbohydrate and nitrogen (N) concentrations, then the degradation rate of Bt-corn stover may be different than that of non-Bt isolines, possibly influencing soil C storage and N mineralization. Thus, this research focused primarily on the comparison of C and N mineralization of corn stover in soil as affected by Bt-trait, plant portion, water-availability and HFC-trait; and secondarily on the existence of Bt-related variations in the chemical structure of corn residues that might affect the degradation rate of stover in soil and consequently the soil C and N dynamics.

A laboratory experiment was conducted under non-limiting N conditions with stover of Bt/non-Bt isogenic pairs of two varieties, a “high fermentable corn” (HFC) line harvested at Snook, Texas and a non-HFC corn line harvested at the irrigated field of Snook and the non-irrigated field of College Station, Texas. The stover was partitioned into three plant portions, incorporated into a Weswood soil and incubated during 223 days.

Results showed that the differences observed in the degradation in soil of Bt vs. non-Bt corn stover were dependent on environmental conditions (irrigated vs. non-irrigated settings) and hybrid variety (HFC vs. non-HFC hybrid lines). The structural composition of corn plants was affected by the Bt-trait, HFC-trait, irrigation and their interactions. Variations in the biomass fractions of the initial stover of Bt and non-Bt hybrids had minimum to non-impact on soil C and N concentrations measured at the end of the 223-day incubation period. Lignin concentration was affected by a Bt-trait*variety interaction. There were no significant differences in lignin concentration between non-Bt/Bt-corn derived stovers of the non-HFC variety irrespective of irrigation regime but Bt-hybrids of the HFC variety contained more than twice as much lignin as the non-Bt isogenic plants. The effects of higher lignin concentration on C mineralization rate appeared to be offset by an increased lignin degradability inherent in HFC-trait.

Overall, results indicated that the cultivation of Bt-modified maize lines is not likely to have significant effects on soil C or N dynamics compared with the cropping of non-Bt hybrids.

DEDICATION

To my unconditionally loving parents, belated María and Nuncio.

To my children, Erica and Javier.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

According to the National Resources Conservation Service (NRCS, 2006), 332 million acres (approximately 14.4%) of the 2.3 billion acres of land in the United States are prime farmland, and only about 20% is sufficiently fertile for crop production. In 2006, about 24% of this fertile soil was planted to corn (*Zea mays* L.), with more than 60% of it being genetically engineered (GE) varieties, including herbicide resistant varieties (21%), insect-resistant corn, usually referred to as Bt-corn (25%) and stacked (insect and herbicide-resistant) gene corn (15%). During the same year, 71.8 million acres of corn for grain were harvested with an average yield of 154.7 bushels per acre (9711 kilograms per hectare) (NASS, 2006a). Considering that, on average, aboveground corn plant dry matter is 50% grain and 50% stover, an equivalent of almost 621.6 billion pounds (2.8×10^{10} kilograms) of corn residue were left on American fields.

Corn stover is mainly composed of corn leaves and stalks, although in practice, stover is everything that is left in the field after harvest, including stalks, leaves, husks, cobs, anchor roots and even kernels of grain that may have dropped from the harvester.

This thesis follows the style of the Soil Science Society of America Journal.

A minimum of 30% of corn residue should be left on the soil surface to diminish erosion (US Code, 2000), the remaining residues may or may not be tilled into the soil according to the region because no-till gives different results in diverse geographical areas (Linden et al., 2000; Mann et al., 2002; Spedding et al., 2004; DeFelice et al., 2006), and therefore, farmers adopt various techniques to deal with the stover. At least 50% of corn stover, roughly 300 billion pounds, will be tilled back into the soil (DeFelice et al., 2006; NASS, 2006b), with about 120 billion pounds of corn stover containing Bt-protein(s). The return of corn residues to the soil is essential to the next harvest's yield and overall soil fertility and productivity (Havlin et al., 1999; Wilhelm et al., 2004). Residue incorporation affects soil properties such as aggregation, water infiltration, water-holding capacity, aeration, bulk density, nutrient availability, pH, cation exchange (CEC), and buffer capacity, etc. Residues incorporated into soil influence the quality and quantity of soil organic carbon (SOC), and emit earthbound greenhouse gases (GHG) that potentially affect global warming (Karlen et al., 1994; Martens, 2000; Trinsoutrot et al., 2000; Torbert et al., 2000; Lal, 2001; Follet et al., 2001; Mann et al., 2002; Flerchinger et al., 2003; Blanco-Canqui et al., 2006). Net soil C balance depends on crop management practices and residue quality. Incorporating stover may result in a decline in soil C if it is not accompanied by rotation with cover crops, reduction of tillage, and proper application and type of fertilizer (Lal, 2001). Increasing soil carbon levels is feasible, but not easily accomplished. Researchers have shown that C sequestration in soils is directly proportional to nitrogen (N) application rates, yet most of the time this increase in soil C content is counterbalanced by CO_2 emissions associated with fertilizer production, transport, and application (Wallace et al., 1990; Green et al., 1995; Gregorich et al., 1996; Vanotti et al., 1997; Alvarez, 2005; He et al., 2006; Bolinder et al., 2007).

Because the energy industry is proposing the use of corn stover for conversion to ethanol as an alternative to fossil fuel, knowledge about the degradation of this residue in soils becomes crucial for soil scientists and extension agents responsible for advising farmers about their best residue management options. This is a difficult matter because enough corn stover must be left in the field to prevent wind and water erosion and a portion should be incorporated into the soil to increase C sequestration and maintain soil quality. The remaining residues may then be used for ethanol production. The subject is further complicated because stover management affects weeds, insects and fungal diseases as well as the chemicals applied to control them, which in turn will affect all the above and impact the producer’s finances.

Past research conducted on the degradation of Bt and non-Bt corn residues in soil has shown dissimilar results. These studies were carried out with different isogenic pairs of Bt/non-Bt maize, on diverse soil textures and under different climatic conditions (Schneffe et al., 1998; Hopkins and Gregorich, 2003; Hopkins and Gregorich, 2005; Dubelman et al., 2005; Blanco-Canqui et al., 2006; Duiker et al., 2006); therefore, no general conclusion can be drawn from them. Thus, the first objective of this research study was to determine whether Bt-corn stover degrades at a slower rate than its corresponding non-Bt isoline of corn hybrids suited for cropping in Southeast Texas’ soil and climate.

Jung and Sheaffer (2004) concluded that there was neither a statistical difference in the lignin concentration of six Bt/non-Bt isogenic pairs, nor any appreciable difference in either performance or quality in terms of grain production or rumen digestibility. Podersimo et al. (2005) showed that 32K61 and 32K64(Bt) corn stover had significantly different xylan and lignin concentrations. Furthermore, it has been reported that Bt maize had a higher (but not significant) N concentration, and a lower concentration of lignin than its corresponding non-Bt isoline (Escher et al., 2000), and also that Bt-corn had higher lignin concentration (Saxena and Stotzky, 2001; Flores et al., 2005) than the non-Bt isogenic corn plants. If Bt hybrids do in fact have higher lignin concentrations, then these stovers may degrade at a slower rate than the corresponding non-Bt isolines because lignin takes longer to degrade than sugars, starches, and other simple organics that are rapidly mineralized by microbes (Sylvia et al., 1999). In addition, lignin is a 3-D heteropolymer that links to hemicelluloses by different types of bonds forming an intricate matrix that surrounds the more easily degradable components (a phenomenon referred to as lignin encrustation), thus preventing or delaying microbial attack (Webster et al., 2000; Hopkins and Gregorich, 2003) and slowing the decomposition process.

Consequently, this study was also designed to learn whether the Bt gene insertion produced, as a ripple effect or non-target, changes in the structural chemistry of *Zea mays* L. Specifically, this study determined if Bt-corn stover that contained a higher percentage of lignin than non-Bt corn stover affected the kinetics of its residue degradation in soils. Furthermore, because the Bt trait induces the synthesis of the insecticidal protein during the whole life span of the plant, it may affect the percent N concentration in plant tissue translating into a different C:N ratio of the stover. Elevated N levels stimulate hydrolases and suppress phenol oxidases and peroxidases that accelerate the rate of stover degradation in soil during the first days, resulting in an overall decrease of both C mineralization and microbial biomass in long-term incubations (Soderstrom et al., 1983; Scott et al., 1998; Gallo et al., 2004), irrespective of the N source (Fenn et al., 1981; Buswell et al., 1982; Reid, 1983). Therefore, the third objective was to determine if production of the Bt protein altered the total N concentration of transgenic plants, which in turn could modify soil C mineralization.

Bt-corn has been genetically engineered to express different insecticidal proteins such as Cry1Ab, Cry1Ac, Cry9C, Cry1F, Cry3Bb1, Cry34Ab1, Cry35Ab1, etc., from the soil bacterium, *Bacillus thuringiensis* (USEPA, 2007). The amount and distribution of such proteins in the plant depend mainly on two factors: 1) the event (the process of successfully modifying and inserting a genetic package into the DNA of another organism), and 2) the promoter (the specific DNA sequence that contains the information that regulates when and how often the gene is transcribed and therefore the amount of protein produced by the plant), which determines where and how much delta endotoxin will be produced throughout the life of the plant. For example, event MON832 refers to the introduction of a glyphosate oxidase (GOX) and a modified enzyme (EPSPS) that confers resistance to the herbicide glyphosate. Event CBH-351 refers to the introduction of the Bt protein Cry9C that confers resistance to European [*Ostrinia nubilalis* (Hübner)] and Southwestern (*Diatraea grandiosella)* corn borers, plus an acetyltransferase (PAT) from *Streptomyces hygroscopicus* that confers tolerance to the glufosinate ammonium herbicide (USEPA, 2007; Agbios, 2008). Different seed companies use different events and promoters, so their hybrids may produce the same (or different) proteins that may have a different distribution in plant tissues. As an example, both event Bt176 and Bt11 are produced by inserting the Cry1Ab gene from the *Bacillus thuringiensis* subspecies *Kurstaki* (Btk) that confers the insect-resistance trait, but have different promoters. Event Bt176 has two promoters derived from the maize plant itself: 1) a pollen-specific protein and 2) a phospho-enol-pyruvate carboxylase that causes the Bt protein to be produced only in the green tissues of the maize plants. Thus, the insecticidal proteins of event Bt176 corn are produced in both the pollen and green tissues. On the other hand, the promoter in event Bt11 maize is the phosphinothricin N-acetyltransferase (PAT) encoding gene from *Streptomyces viridochromogenes* (extracted from the cauliflower mosaic virus 35S gene) that causes the Bt insecticidal protein to be produced in all tissues of the plant (Agbios, 2008).

As of now, there is no technology to control Bt gene location (which chromosome and what part of the chromosome) at the time of insertion, and therefore, the location and the amount of the endotoxin produced by the corn plant are not completely known. Thus, the study of soil degradation of Bt proteins per se, or pure protein, is of no interest to this research. Instead, the focus of this study is on the distribution of total N concentration in the different portions (upper, middle, lower) of Bt/non-Bt isogenic corn hybrids and its possible effects on the rate of decomposition in soil of the corresponding stover (Agbios, 2008).

Li et al. (2003) found that when lignin concentration was reduced by repressing a single lignin gene (4-CL), cellulose concentration increased. Boudet et al. (2003) reported instances in which manipulation of lignin genes resulted in modification of the lignin chemical/spatial structure instead of the expected decrease in lignin quantity. Hopkins et al. (2001) reached the conclusion that modifications to lignin quantity as well as composition and conformation of the lignin subunits affected residue degradability in soils and digestibility in animals. Kumar and Goh (2000) studied the decomposition in soil of a variety of crop residues as well as compost and dairy sludge, and determined that, on average, soluble C had a half-life of several hours, hemicellulose and cellulose exhibited a half-life of several days, and lignin showed a half-life of about a year. Furthermore, Kumar et al. (2006) carried out static incubation experiments to study the degradation of Bt and non-Bt residues of spring canola (*Brassica napus* L.), rice (*Oryza sativa* L.), and tobacco (*Nicotiana tabaccum* L.) in soil. They found that after 30 days of incubation, Bt residues of the three crops mineralized between 22 and 27% less N than the corresponding non-Bt residues, although there were no differences in cumulative N mineralization at 45-, 60- and 90-day incubation periods. The authors concluded that in their experiments, lignin concentration in the residues was related to the variability of N mineralized at day 30. However, in a two-year study by Mungai et al. (2005), no consistent differences in the composition of Bt and non-Bt corn residues were observed. They determined that the stem tissue of the hybrid M-00112Bt had a higher lignin concentration than similar tissues of the non-Bt isoline, but the stem tissue of the 33P67Bt hybrid had lower lignin content than the stem tissue of the corresponding non-

Bt isoline. Soluble sugars are preferentially degraded by soil microorganisms, followed by hemicellulose, cellulose, and lignin. Martens (2000) stated that a higher content of lignin is desirable in terms of soil C sequestration because it increases the C content of the more recalcitrant pool. However, it is not desirable for livestock or for the biofuel industry for which the seed companies have already developed hybrids, known as HFC (high fermentable corn) and HES (high extractable starch corn), which have a higher concentration of soluble C (Boudet et al., 2003; Ragauskas et al., 2006).

Soil C sequestration potential is the resulting balance between C inputs and outputs (Lal, 2001). Stover constitutes the main input in cornfields, so its decomposition rate in soils is a primary determinant of C sequestration and N availability (Marrs et al. 1983). Structural and non-structural plant residue components are important predictors of potential decay rates (Trinsoutrout et al., 2000). However, the degradation kinetics in soil depend on many other factors such as climatic conditions, crop management, availability of exogenous nutrients (Melillo et al., 1982), and soil physical and chemical characteristics (Wright and Hons, 2004). All of these influence the composition and activity of microbial communities, which ultimately determine the rates of plant residue breakdown and nutrient release (Martens, 2000), and modifies the quality and quantity of N and C that accumulates in soil, and therefore, the fertilizer requirements for the next crop. In consequence, the last objective of this research was the comparison of the rate of degradation in soil of stover from different portions of Bt/non-Bt isogenic corn plants with the rate of degradation of equivalent portions of Bt/non-Bt high fermentable corn varieties of plants grown at the same location to prevent the possible masking of the Bt effect by environmental conditions.

OBJECTIVES

The main objectives for this study were:

1. To determine the effect of Bt protein insertion on the decomposition rate in soil of stover obtained from three different portions of corn plants grown at two different locations.
2. To learn whether Bt gene insertion was associated with changes in the structural chemistry of corn plants, i.e. did Bt-corn stover from any of the three different portions of the plants grown at two different locations have a higher percentage of lignin and/or N than stover from the equivalent portions of the isogenic non-Bt plants grown at the same or different location.
3. To observe if the stover of three different portions of Bt-corn plants had a different N-mineralization rate than the corresponding portions of the non-Bt isogenic plant-derived stover that could be attributed to an increased N concentration because of the production of the Bt-protein.
4. To compare the degradation rate in soil of stover derived from three different plant portions of Bt/non-Bt isogenic corn hybrids vs. the same plant portions of isogenic Bt/non-Bt high fermentable corn hybrids grown at the same location.

CHAPTER II

LITERATURE REVIEW

GREENHOUSE GASES AND AGRICULTURE

Soil organic carbon (SOC) represents the largest C reservoir in interaction with the atmosphere. The depletion of the SOC pool enhances atmospheric CO_2 concentration while carbon sequestration transfers atmospheric CO_2 into the terrestrial C pool. In the past, agriculture was the main cause of the increasing CO_2 concentration in the atmosphere, but at present the combustion of fossil fuels is the main cause, being responsible for about 75% of greenhouse gases (GHG) emissions (USEIA, 2007). Actually, the predominant components of terrestrial sequestration include soil, biota and biofuel (IPCC, 2007; USEIA, 2007), since corn stover is being proposed as feedstock for bioethanol production, the management of the millions of tons of corn residues left every year on the fields will have a large impact on these three elements.

The dynamics of soil C involved in the degradation of corn residues are directly related to CO_2 emissions, while soil nitrogen (N) dynamics and the management of N to match crop needs may increase or reduce N_2O emissions and have adverse effects on water quality (Lal, 2001). Thus, any possible effect of the Bt-trait on corn stover decomposition in soils and its associated N and C dynamics needs to be carefully assessed and considered because it may influence global climate change both directly through the emission of GHG, and indirectly because it influences soil fertility and consequently the amount of stover that could be sent to ethanol production.

In the US, agricultural-related activities released about 7% of the country's total GHG emissions during 2005 (USEIA, 2007). Livestock-related processes were the main source of CH_4 emissions and a very small source of N_2O. Conversely, agricultural soil management activities were the largest source (78%) of US total N_2O emissions, and contributed a minor amount of CH_4 that came from rice cultivation and field burning of plant residues (USEIA, 2007). Synthetic fertilizer applied to forests and agricultural soils contributed to direct release of N_2O, and wildfires and crop residue burning caused the emission of CO_X, NO_X, N_2O, CH_4, SO_X, volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs) (Haysa et al., 2005).

Other soil management activities, such as irrigation, drainage, tillage practices, and fallowing of land, can also affect fluxes of N_2O, as well as soil C and fossil fuel CO_2 emissions. Lastly, seasonal temperature and precipitation changes, as well as regional climate variability, influence the rates of both soil N and C emissions.

CARBON DYNAMICS IN AGRICULTURAL SYSTEMS

The overall C budget of an agricultural ecosystem has one input, photosynthesis, and several outputs, including SOM oxidation or erosion, fossil fuel burning, microbial and plant respiration and deforestation.

Carbon Dioxide

Carbon dioxide outputs from agricultural systems may originate from several sources including plant respiration; microbial respiration due to surface and incorporated (tilled) crop residues and soil organic C decay; chemical reactions involving existing (pedogenic) or applied carbonates (liming); fossil fuel burned to operate machinery (tractors, harvesters, etc.) and irrigation systems; and CO_2 released by fossil fuel used to manufacture fertilizers, lime, growth regulators and other agricultural-related industrial operations. The rate of soil CO_2 emission is controlled by soil conditions such as temperature, moisture, pore size and structure, SOC concentration and quality, the CO_2 concentration gradient between the soil and the atmosphere and climatic conditions such as wind speed (Reich et al., 2001). Agricultural practices such as tillage and residue management also impact soil CO_2 emissions.

Carbon dioxide is captured by plant photosynthesis and rainfall deposition. In the atmosphere, CO_2 dissolves in rainwater to form carbonic acid (H_2O (aq) + CO_2 (g) + H_2CO_3 (aq)). That is why rainwater has a slightly acidic pH of 5.6. Carbonic acid is too weak to dissociate much at $pH < 5$ but has significant acidifying effect in alkaline and neutral soils where it can release plant nutrients and promote mineral weathering. In addition, the slightly acidic percolating water gradually replaces and leaches soluble exchangeable cations (calcium, magnesium, potassium, and sodium) out of the soil profile, acidifying the soil in the process. Thus, acid soils tend to occur in temperate and tropical areas where rainfall is substantial. Conversely, alkaline soils tend to occur in arid and semi-arid landscapes where evapotranspiration exceeds rainfall, which favors retention and accumulation of exchangeable cations.

Carbonates

Atmospheric CO_2 sequestered in the form of inorganic C in soils occurs as carbonates in the calcic and petrocalcic horizons where it was geomorphically cemented or in soils where it was transported and accumulated over the ages. Naturally occurring soil carbonate consists of two pools: geogenic (detrital limestone particles) and/or pedogenic (in situ precipitated $CaCO_3$). Early forms of pedogenic or in situ carbonates include calcified root hairs, fungal hyphae, needle-fiber calcite, and micro-peloids (Serna-Perez et al., 2006).

When these stable carbonates are carried up to the soil surface by naturally occurring or anthropogenic-mediated processes and exposed to the elements, they may undergo dissolution and liberate CO_2 back into the atmosphere. It is known that the trapping of atmospheric CO_2 and its deposition as carbonate is an ongoing process in arid and semiarid soils where it accumulates under dry climatic conditions until it is removed by erosion. On the other hand, in wet climates, rainfall will most likely dissolve carbonates in and at the soil surface, releasing cations (usually Ca^{++}, Mg^{++}, and Na^{+}) that may be translocated deeper into the profile or leached out with percolating water. Carbonates that are added to acidic soils in the form of lime or dolomite to ameliorate soil acidity may undergo similar reactions depending on soil drainage and weather conditions, or they may be dissolved by soil acidity (HNO_3) releasing CO_2 in the process (USEIA, 2007; Serna-Perez et al., 2006).

When added to soil, Ca^{++} and/or Mg^{++} dissolved from liming materials displace hydrogen (H^{+}) from clay particles. The displaced hydrogen then reacts with carbonate, reducing soil acidity. Carbonate dissolved from the limestone materials will produce carbonic acid that is not stable under this soil condition and will dissociate into CO_2 and water:

H^{+} - clay + $CaCO_3$ (limestone) → Ca^{++}- clay + H_2CO_3 (carbonic acid) → $H_2O + CO_2\uparrow$

Methane

Methane is released from a number of natural sources such as wetlands, landfills, sewage, guts of termites and wildfires. Agriculture-related CH_4 gas emissions derive from livestock and domestic ruminants, open burning of crop residues, and from ever-increasing rice cropping areas (USEIA, 2007). Ruminants including cattle, sheep, goats, camels, and buffalos have a unique four-chambered stomach. Methane is generated in one of these chambers, the rumen, where bacteria break down cellulase and generate CH_4 as a by-product. The rate of CH_4 production depends on animal species, individual animal characteristics such as body weight and age, quality and quantity of feed, exercise, etc. (USEIA, 2007).

Biomass open burning produces CH_4 together with a variety of other gases. Emissions from this source depend on type, quality, and quantity of crop residues burned, burning conditions including temperature, quantity and quality of fuel and burning efficiency (Jenkins et al., 1996). Flooded rice fields provide ideal conditions for CH_4 emissions, including oxygen depletion (anaerobiosis), excess water and high organic substrate for the development of anaerobic CH_4-producing (methanogenic) bacteria that decompose the organic matter originated by rice senescent leaves and harvest residues, dead weeds and algae, green manure, and compost (Havlin et al., 1999.) About 90% of the CH_4 produced by methanogenic bacteria is oxidized back to CO_2 by CH_4-consuming (methanotrophic) bacteria. A small portion of the remaining CH_4 is transported into the atmosphere by diffusion and bubbling through floodwaters, and the rest is carried to the surface by rice plants themselves. The minor amount of CH_4

that is dissolved in water may exit the system by leaching (Neue, 1993). Rice cultivation is considered the main global source of CH_4 from agricultural sources (IPCC, 2007).

NITROGEN DYNAMICS IN AGRICULTURAL SYSTEMS

The N budget of an agricultural system can be represented as follows: organic, or indirect N, in the form of plant residues and animal manure plus inorganic N added by biological and atmospheric N_2 fixation and rainwater deposition minus the N that leaves the system when plants are harvested and what is lost by volatilization, leaching, runoff, and erosion (Sylvia et al., 1999). Inorganic N may be introduced to soils by:

a) Biological atmospheric N_2 fixation. Nodule-forming *Rhizobium* bacteria inhabit the roots of leguminous plants, and through a symbiotic relationship convert atmospheric N_2 to NH_4^+ making N available to the leguminous plants in exchange for C (energy). The NH_4^+ that remains in the soil after the death or harvest of the legumes, plus the N mineralized during the microbial decomposition of the crop residues left in the soil, will benefit other plants grown at the site (Havlin et al., 1999; Sylvia et al., 1999). Perennial legumes, such as alfalfa, can fix several hundred pounds of N per acre per year (Burity et al., 1989).

b) Rainfall deposition. Rain will wash down nitrates from the surface of foliage, nitrates that were formed in the atmosphere by lightning-mediated fixation of atmospheric N_2 and, those released by industrial activities. It has been calculated that rainfall deposition can add as much as 11 kilograms of N to the soil per hectare per year (Krusche et al., 2003). The nitrogen oxides and NH_4^+ that are washed to earth are formed during electrical storms, by internal combustion engines and through oxidation by sunlight (Sylvia et al., 1999).

c) Inorganic N fertilizers.

Ammonium may be assimilated by plants or be oxidized by bacteria (*Nitrosomonas*, *Nitrosococcus*) to nitrite and then (*Nitrobacter*) to nitrate, which is generally the preferred form absorbed by plants. Both NH_4^+ and NO_3^- are temporarily unavailable to plants when they are immobilized, that is, converted into microbial tissue. When microorganisms die, the bacteria and fungi that consume them mineralize and nitrify the organic N back into NH_4^+ and NO_3^- (Sylvia et al., 1999).

The timing of this immobilization is a major concern for farmers, especially for crop residues that contain high amounts of C in relation to N such as corn stalks, as microbes need more N to digest the material than is present in the residue. Although there will be a net gain after the microbial immobilization-mineralization processes have taken place, it may not be available when next crop plants need it the most.

Outputs

Substantial amounts of N are lost from the soil system through crop removal. For example, a corn crop removes an estimated average of 220 kg N per hectare for a grain yield of 9400 kg per hectare (150 bushels of grain per acre), thus, crop removal accounts for the majority of the N that leaves the soil system (Havlin et al., 1999). Direct N_2O emissions from agricultural systems vary highly depending on the combination of many factors such as rate, type, and form of fertilizer application, weather conditions, soil chemical and physical properties, residue management, etc. If the soil is not sufficiently aerated, facultative microorganisms will shift from aerobic to anaerobic respiration and use soil NO_3^- as an alternate electron acceptor, reducing nitrate to nitrite (NO_2^-), and gaseous nitric oxide (NO), nitrous oxide (N_2O) and ultimately N_2, that will be lost from the soil into the atmosphere (denitrification). In poorly aerated soil, such as compacted or flooded soils, denitrification can occur within two or three days of application of nitrate-type fertilizers and can result in large loses of gaseous N, particularly under warm temperatures (Gupta et al., 1994; Sylvia et al., 1999). Leaching losses of N occur when soils have more incoming water (rain or irrigation) than the soil can hold. As water moves through the soil, NO_3^- in the soil solution is moved downward by water. Nitrogen loss by leaching becomes important in relatively coarse, sandy soils. Ammonium will be held by an ionic bridge to the negative sites on the clay in soil; therefore, these ions are less likely to leach (Sylvia et al. 1999; He et al., 2006).

In alkaline soils, NH_4^+ may be converted ($NH_4^+ \rightarrow NH_3$ (gas) + H_2O) to gaseous NH_3 and lost (volatilized) to the atmosphere through ammonia volatilization. Soil conditions including moisture content, texture, cation exchange capacity (CEC), pH and residue cover will affect the rate and amount of NH_3 loss. Ammonia volatilization is most likely to take place when soils are moist and warm and urea or NH_4^+ is on or near the soil surface (Jenkinson et al., 1985; Sylvia et al., 1999; Kumar and Goh, 2000). It seems obvious then that minimizing the time the N is in the soil before plant uptake would also cut down the time when conditions are favorable for losses. This is easier said than done, as the optimal rate and timing of N application in corn is difficult to achieve. In spite of the many efforts and management techniques developed, researchers have calculated that about half the N fertilizer applied to corn is wasted by denitrification, leaching, and/or volatilization (Vanotti et al., 1997; Vetsch and Randall, 2000; Kumar et al., 2006).

By way of illustration, maize requires high N inputs shortly after planting for good root development. As much as 40 percent of the total N taken up by the corn plant occurs from silking onwards. When all (NT) or part of the crop residues are left on the soil surface after harvest, farmers will usually add 44 to 100 kilograms of N per metric ton of corn stover left on the field to avoid microbial N immobilization insuring timely residue decay and availability of mineral N forms when the plants need it (Al-Otayk et al., 2008; Donner et al., 2008). However, when urea is sprayed on corn stalks and there is little soil water to absorb it, most of the applied N may be lost by volatilization (Frederick et al., 1990; Vetsch and Randall, 2000). Enough water (rain or irrigation) is needed at this time to wash the urea from the residue so that it will be in direct contact with the soil, but if rainfall is high during the next weeks, N may be lost by denitrification, leaching, or both (Frederick et al., 1990; Green et al., 1995; Gudelj et al., 2005).

LAND MANAGEMENT

Soils represent the main reservoir of C in the terrestrial biosphere. A large portion of this C has been lost due to land clearing and extensive tillage and has potential to worsen as world population increases. Thus, protecting and recapturing C in soils becomes a priority and the need to increase C sequestration in soils fosters the implementation of land-use changes (LUC) and improvement of land management practices (LMP). Changes in land use and management practices include: shifting from mono-cropping patterns to mixed cropping systems; agro-forestry and tree crop plantations; conservation tillage and mulching; efficient water management; modification of the timing and placement of organic and inorganic inputs; manipulation of soil fauna; livestock management (improved feeding, dietary additives, breeding, etc.); grazing land management (modified grazing intensity, introduction of different species, nutrient and fire management); and manure management (storage, handling and anaerobic/aerobic digestion techniques). These procedures mitigate climate change by modifying the rate and direction of C (also N) fluxes between the atmosphere, terrestrial vegetation and soil, and at the same time improve soil fertility, recover degraded lands, and enhance the long-term sustainability of farming (Lal, 2005; Lal, 2006).

Usually, the best LMP involve the implementation of more than one new or improved technique. As an example, agricultural intensification, considered by researchers as the best alternative in most agro-ecosystems, is the combination of integrated nutrition management, mixed cropping practices and efficient use of external inputs (IPCC, 2007). LUC and LMP have the potential to introduce positive transformations, however, the complex network of interrelated effects among the different practices requires a careful analysis to insure a successful outcome as one practice may offset the benefits of another, or the end result may turn out to be negative. For instance, the emission of CH_4 by cattle should be considered when pastures are increased, and the release of N_2O from N fertilizers must be taken into account before their application. Rice culture presents a particular challenge because N-fertilizers applied to improve yields and soil organic matter also increase the emissions of CH_4 (has a greater greenhouse effect than CO_2) (Neue, 1993).

LUC and LMP implementation means that there will be changes in GHG emissions and soil C stocks; however, the final net C budget variation may be a shortfall. Such could be the case with practices that successfully improve crop yield and agriculture productivity at the expense of existing forests or virgin pastures. It has been calculated that when grassland or forest is cleared and cultivated for agricultural production, there will be an estimated average loss of 8000 kg C per hectare from SOC over a 20-yr period (Lal, 2004), equivalent to 400 kg C per hectare of SOC per year. The majority of C usually is lost during the first five years after clearing and tillage, and calculations did not include C-CO_2 lost in the manufacturing of agricultural inputs, transport, and use of various deforestation and agricultural equipment. Conversely, when cultivated land is abandoned and allowed to revert to grassland or forest, an average accumulation in soil of 335 kg C per hectare per year may be achieved (Tubiello et al., 2007), and there will be no collateral C-CO_2 losses due to the use of machinery and agricultural inputs.

The combination of deforestation and agricultural cultivation has caused large losses of SOC in many areas of the world. About 57% of the original organic matter content was lost from Georgia soils (southeastern USA) during a period of 25 years (Giddens, 1957), and similar land use changes in the Chiapas region, Mexico, caused the release of 20 million tons of C to the atmosphere between 1970 and 1990 (De Jong et al., 1999). During 1991, deforestation practices in Brazil, Colombia, Costa Rica, Mexico, Peru, and Venezuela were accountable for the combined emissions of 419 million tons of C. It was estimated that the deforested lands in Mexico released as CO_2 an average of 230 metric tons C per year per hectare of deforested land while forests in Peru released about 9 metric tons C per year per hectare of forest (IPCC, 1996). By 1993, about 43% of the forestlands of the Amazon Colony, Brazil, had been deforested for slash and burn agriculture and pastures for cattle production (Fujisaka et al., 1998). A site-specific and yet holistic approach is required to determine management measures that address environmental priorities while ensuring financial viability for the land owners, minimizing on-site and off-site effects like erosion and chemical transport, etc., and matching adequate biomass/crops to increase the sustainability of intensive production systems. All of the above should be adjusted periodically to match changing climatic conditions.

The early SOC loss that occurs at the time of site establishment when soil surfaces are kept clean-tilled may be compensated or avoided by growing cover crops to avoid C and N losses (Torbert et al., 2000). The no-tillage (NT) system used with cover crops provides protection against wind and water erosion, affects water infiltration, soil temperature, aeration and structure, nutrient cycling, weed control, microfauna diversity and abundance, root penetration and seedling emergence (Reicosky, 1997; Torbert et al., 2000). As good as NT sounds, it may not always be the best choice. No-till corn practices, which proved to be useful and profitable for the Corn Belt region (Wright and Hons, 2005), delayed the rise of soil temperature in Minnesota, impeding germination and early crop growth, and increasing the potential for insect and disease damage

(Reicosky, 1997; Vetsch and Randall, 2004). Under cool and wet conditions, and especially in poorly drained soils, corn has a low tolerance to high levels of crop residue on the soil surface, so that continuous corn in this region and climate requires tillage for residue incorporation. In addition, in poorly drained, non-leveled, high-rainfall areas, sedimentation and nutrient losses are of concern (Isse et al., 1999; Blanco-Canqui et al., 2006; DeFelice et al., 2006). Consequently, it may be necessary to implement conservation methods other than tillage to reduce sediment losses; or introduce changes such as switching to corn-soybean [*Glycine max.* (L.)] rotation (that would also allow NT), if climatic conditions in the region favor profitable soybean growth.

This situation is well illustrated by the results of studies carried out on corn-soybean cropping systems in the upper mid-west USA where no significant C storage was evident under NT. The authors concluded that for that particular region and climate, corn-soybean cropping required at least some tillage to sequester C in soil and suggested that a long-term biennial chisel plowing would offer the benefits of improved C storage without compromising yields as compared with those obtained under intensive annual tillage (Vetsch and Randall, 2004; Venterea et al., 2006).

RESIDUE MANAGEMENT

Crop Residue Management (CRM) refers to all practices that deal with crop residue left on the soil surface. These include residue amount, quality, spatial distribution, thickness, and geographical orientation, crop rotation, use of cover crops, tillage type, intensity and depth, number of passes over the field by agricultural equipment, etc., with the objective of sequestering C, diminishing GHG emissions, improving soil fertility and crop productivity (USDA, 1999). The amount of C stored in soil is a function of total inputs, the rate of biomass decay and SOM turnover. Soil organic C may also be respired and returned to the atmosphere if cultivation intensity increases (Franzluebbers and Follett, 2005). Both decreased or increased yields may lead to a negative net C flux. More absolute quantities of C will be lost by increased than by decreased yields (West and Marland, 2003). Critical variables to be considered when assessing proper residue management of each specific site are usually climate, clay content, drainage, and slope. For example, leaving 30% of the crop residues on the soil surface is generally enough to ensure protection from erosion, however, if there is excessive slope, it may be necessary to leave up to 70% of the crop residues on the soil surface to obtain the same results (Wilhelm et al., 2004).

The response of each particular site to modifications in crop residue inputs is the result of the interactions of the critical variables and many other factors including land management and history of land-use, residue quantity, chemical composition, C/N ratio, lignin content, the size of residue pieces, tillage, cover crops, and fertilizer use (Wallace et al., 1990; Green et al., 1995; Gregorich et al., 1996; Vanotti et al., 1997; Follett et al., 2001; Alvarez, 2005; Wright and Hons, 2005; He et al., 2006; Bolinder et al., 2007). Changes in crop management systems can modify soil structure and have a major influence on water and air movement, biological activity, root penetration, seedling emergence, water-holding capacity, and availability of nutrients. No tillage in conjunction with cover crops minimizes soil degradation, promotes beneficial changes in the chemical, physical and biological properties of the soil, diminishes external chemical input needed and promotes higher biodiversity and sustainability (Reicosky, 1997; Reicosky and Forcella, 1998; Torbert et al., 2000; Vetsch and Randall, 2004). Furthermore, future innovations in residue management practices will likely influence nutrient cycling, water infiltration, sediment retention, nutrient availability, soil compaction (Weil and Magdoff, 2004), and greenhouse gas emissions (Follett et al., 2001; Franzluebbers and Follett, 2005). Even subtle modifications may affect the soil microbial biomass, N mineralization potential, particulate organic matter (Franzluebbers et al., 2001), the number and composition of soil fauna, and the number of pest and beneficial organisms (Govaerts et al., 2008). In turn, the response of soil organisms to modifications of the soil environment will induce changes in soil physical and chemical characteristics and crop performance (Wright and Hons, 2005).

Land Management History

No till cultivation can lead to a large reduction in net CO_2 emission because it reduces the rate of decomposition of both the native SOM and crop residues. The rate of residue decomposition is decreased because there is less biological activity on the soil surface than in the soil, and SOM turnover is slower because the soil is generally less aerated and the organic compounds inside the aggregates are not put in contact with the microbes as when broken by plowing (Lal et al., 2007). Repeated moldboard plowing allows rapid CO_2 loss and O_2 entry because it loosens the soil and inverts both soil and residue. Much of the CO_2 released initially is from entrapment and storage, and the remainder from accelerated microbial respiration when O_2 entry is accelerated. Long-term plowing accelerates microbial decomposition in the 15 to 30 cm layer and the lack of shallow residue causes aggregate breakdown, which ultimately decreases surface soil C content (Reicosky and Allmaras, 2003).

The maximum amount of SOC that a particular soil is able to hold, and the time that it takes to reach a new steady state or saturation point, is the result of interactions of all the aforementioned factors (Hooker et al., 1982; Franzluebbers et al., 2001). Once a soil has reached the saturation point, it cannot hold any more C (as SOC), and will release back into the atmosphere whatever additional organic C is added (Six et al., 2002). When cropland residue management shifts from conventional tillage (CT) to NT, the rate of increase in SOC may be relatively low during the first 3 to 5 years. It will generally peak in 5 to 10 years and will reach a steady state in 20 to 50 years following the tillage management change. However, the SOC gained with the change will rapidly be lost if CT is re-introduced (Franzluebbers et al., 1996; Franzluebbers et al., 2001; Reicosky and Allmaras, 2003).

Finally, on evaluating a change from CT to NT, it should also be considered that the benefits of a decrease in the use of fossil fuels because of fewer passes of agricultural equipment may be offset by an increase in the use of N fertilizer and lime, and its associated emissions of N_2O (assuming there is no change in productivity because of the shift in tillage practices) (Follett et al., 2001; Lal, 2001).

Residue Quality-Quantity

Residue incorporation will affect soil properties such as aggregation, water infiltration, water-holding capacity, aeration, bulk density, nutrient availability, pH, cation exchange (CE) and buffer capacity (Karlen et al., 1994; Martens, 2000;

Trinsoutrot et al., 2000; Torbert et al., 2000; Lal, 2001; Follett et al., 2001; Mann et al., 2002; Flerchinger et al., 2003; Blanco-Canqui et al., 2006). Residue incorporation may cause a decline in soil C if it is not accompanied by adequate agricultural practices such as proper fertilizer application, reduction of tillage, etc. (Lugo et al., 1986; Cerri et al., 1991; Izaurralde et al., 2000; Conant et al., 2001; Zan et al., 2001; Lal, 2004). Clapp et al. (2000) showed that residue incorporation and biological activity decreased bulk density within the upper 20 cm. The incorporation of stover into the soil, or tillage, accelerates soil CO_2 emissions because it increases microbial and crop residue contact, enhancing the availability of nutrients and O_2 at the same time. Depending on tillage depth, it may also affect new soil C, or new and relic (stabilized) soil C (Havlin et al., 1999; Wright and Hons, 2004; Wright and Hons, 2005). On the other hand, the removal of crop residue from fields will negatively impact the rate of SOC sequestration as well as compaction, temperature and water retention of soils, and grain and stover production of the next harvest (Blanco-Canqui et al., 2006).

A long-term field experiment in a semi-arid area of Mexico where corn and wheat (*Triticum aestivum*) were grown under rain fed conditions, showed that residue retention caused an increase in beneficial soil microflora populations, and that the effects of removing corn crop stover were more detrimental to soil microflora than removing wheat residues from the fields (Govaerts et al., 2008). That is, the residue composition affected the composition of the microbial community, and the change in the community as residue degradation progresses alters the rate of decomposition. Qualitative differences and type of residues are important. For example, high lignin content is beneficial for C accumulation and soil roots are easily transformed into organic matter, reducing soil compaction, and increasing macroaggregate formation and stability (Eck and Stewart, 1998, He et al., 2005; Wright and Hons, 2005; Johnson et al., 2007). The partition of C between above and belowground zones can be manipulated by the introduction of appropriate species (Lal et al., 2007). No tillage might also be combined with mulch farming or cover crops in order to be more effective (Lal et al., 2007). These practices largely depend on water availability.

Tillage

The major concern in relation to tillage is the elevated CO_2 loss from the soil observed immediately after tillage as aggregates are broken liberating the CO_2 trapped inside. The rest of CO_2 that leaves the soil is the result of increased soil C-microbial contact and enhanced O_2 entry caused by soil overturning (Reicosky, 1997; Schlesinger, 1999). The magnitude of CO_2 loss depends on frequency and intensity of soil disturbance caused by tillage operations. Tillage fragments soil aggregates, enhances the release of soil nutrients for crop growth, kills weeds, and modifies the circulation of water and air within the soil. Tillage affects the CEC and water holding capacity, SOC, water/solutes, gas content and composition (Reicosky and Allmaras, 2003). Intensive tillage can adversely affect soil structure and cause excessive break down of aggregates, leading to potential soil movement via water/wind erosion. Intensive tillage accelerates SOC loss and greenhouse gas emissions that affect environmental quality.

The type of tillage used depends mostly on crops and crop rotations that are chosen according to site-specific topography, climate, soil structure and other required conditions for optimum crop growth and economical profit. Although tillage effects on soil CO_2 emission are complex and often vary (DeFelice et al., 2006 ; Drury et al., 2006; Duiker et al., 2006), conservation tillage is regarded as one of the most effective agricultural practices for enhancing SOC sequestration and reducing CO_2 emission from agricultural soils (Reicosky, 1997). Conservation tillage is a relative term meaning the combination of tillage and other residue practices that will protect soil resources while maximizing crop yields within the possibilities of each specific land-site (USDA, 1999). The definition of conventional versus conservation tillage depends also on the geographic location and specific soil conditions (Clapp et al., 2000; Reicosky and Allmaras, 2003). As an example, in most circumstances, 30% of crop residues remaining on the surface after planting will be enough to protect the soil from erosion (Wilhelm et al., 2004).

Every tillage type has advantages and disadvantages that need to be carefully analyzed before they are implemented. For instance, in crops grown without irrigation in drought-prone soils, reduced tillage allows more efficient water use that can translate into higher yields, reduced soil compaction and saving of time and fuel (Franzluebbers et al., 1996; Franzluebbers et al., 2001). However, in wet climates and especially in poorly drained soils, the same type of tillage is likely to cause compaction, flooding, and delays in planting because fields are too wet or too cold to plant, and may carryover diseases or pests in the crop residue that will negatively affect the crop to be planted. Additional problems may be allelopathy (the repression or destruction of plants from the effect of certain toxic chemical substances produced and released by other nearby plants) as can be seen when lettuce is planted directly into rye residues. Conservation tillage decreases evaporation, increases infiltration, diminishes fuel, labor, and machinery requirements, and improves timing of planting and harvesting. The plant residues left on the soil surface increase soil fauna, mainly decomposers, affecting the food web by stimulating bacteria-fungi, microarthropods and nematodes, and macroarthropods (earthworms, ants, etc.) (Hendricks et al., 1986). Thus, it has been proposed that long-term zero tillage with residue retention creates favorable conditions for antagonists and predators of pathogens that results in an environment more antagonistic to pathogens. However, the residue that remains on the soil surface also diminishes the soil temperature-increase rate, which in temperate and cold climate regions impedes germination and early crop growth, and increases the potential for insect and disease damage to crops.

By way of illustration, Arizona, California, and Texas have plow-down laws requiring that cotton plants be disposed of to eliminate the overwinter food source for bollworms and boll weevils. Thus, conventional tillage is a common procedure in cotton crops grown in these states. Cabbage yields under CT were 65% higher than under NT that delayed planting a month because the site was too wet to plant. Furthermore, under relatively warm and wet climatic conditions, NT corn resulted in delayed planting, poor emergence and uneven stands that reduced yields by 6 to 22% in Wisconsin and Illinois (Nafziger et al., 1991). While reduced tillage and SOM buildup contribute to stable soil structure deep into the soil profile, this undisturbed structure also produces macropores and preferential flow channels that can direct nutrients, even phosphorus, downward into deeper parts of the soil profile. Conversely, reduced tillage limits chemical and organic fertilizer application to surface positions that builds up a stratified layer of crop nutrients that concentrate on or near the soil surface becoming vulnerable to rainfall, runoff, and wind events that can remove these highly important substances from the soil surface.

Conservation tillage systems that leave crop residues on the soil surface in wet, heavy soils often face compaction problems (Lal et al., 2007). One of the many solutions developed would be to implement strip tillage that allows an efficient planting and good growth of plants in the loosened soil of the tilled strips while untilled parts conserve soil and water, and might help in controlling weeds. Strip-till is a conservation system that combines the soil drying and warming benefits of conventional tillage with the soil-protecting advantages of NT by disturbing only the portion of the soil to be seeded, with the added advantage that chemicals and fertilizers can be applied during tillage operations.

However, strip till requires multiple trips thereby increasing compaction. Soil compaction cannot be eliminated but it can be managed by separating traffic zones from cropping zones within a field as part of controlled traffic techniques that also eliminate overlaps and skips during seeding and fertilizer and pesticide applications, saving 10 to 15% of chemicals and seed. Controlled traffic also improves traction, flotation, and timeliness of planting, spraying, and harvesting while minimizing potential yield losses from compaction (Reicosky and Allmaras, 2003; Lal et al., 2007). When soil has been heavily compacted, it may be necessary to deep or shallow-rip soils to restore pore space and permeability and reduce run-off. In humid climatic regions, compaction damage is virtually unavailable. Deep-ripping will increase soil drainage by opening up the soil and allowing water to infiltrate at a faster rate that helps reduce erosion by getting the water away quicker. Shallow-ripping allows preparation of the ground without inverting the fertile topsoil. In dry and cold climates, moisture issues may be diminished by ridge-tillage practices that improve infiltration, enhance soil moisture storage by reducing evaporation and preventing runoff, and harvest water from snow during the winter. In ridge tillage, plants grow on a bed or ridge, and the ridges are a product of cultivation of the previous crop and are not tilled out after harvest. As an example, cotton is many times grown on ridges for irrigation purposes, and soybeans are ridge-tilled to decrease the amount of herbicides used to control weeds.

While the intended result of tillage is usually the elimination of weeds, the weed-stimulating aspect is equally important. The specific factors that stimulate weed seed germination may involve combinations of light, temperature, scarification, physical displacement, O_2 potential, and available N. An early spring tillage pass will eliminate present weeds and bring on a new flush of weed seed germination. Ridge tillage avoids early season soil disruption and the stimulatory effect it has on weeds. Different environments will have different optimum conditions for different species of weeds that will be affected, negatively or positively, by the type of tillage chosen. For example, foxtail (*Setaria faberi Herrm*.) germinates best at shallow depths and presents a great problem in NT, while velvetleaf (*Abutilon theophrasti Medik*.) has long-lived seeds that create concern in systems where tillage first buries seed and then unearths them. Studies have shown that ridge tillage resulted in low numbers of both foxtail and velvetleaf in the absence of herbicides (Buhler and Daniel, 1988).

Cover Crops

Conservation tillage usually must be combined with plant cover or mulch management to be effective. The quantity and type of mulch/cover crop depend on site-specific conditions (Reicosky and Forcella, 1998; Isse et al., 1999; USDA, 1999; Flerchinger et al., 2003). Cover crops tend to be a better option than mulch, especially when left on the field, as they provide simultaneous above and belowground benefits. Produced in rotation after harvest, cover crops may add up to one ton of C per hectare per year to the soil C stock (Isse et al., 1999; Lal et al., 2007). Greater surface accumulation of crop residues in reduced and NT systems tends to slow decomposition of N-poor residues such as cereal straw. If the stover is incorporated close to the time of high crop demand, N supply during that season can be reduced by immobilization. Immobilization will also decrease the efficiency of N-fertilizers applied on the straw residues. Thus, in addition to the environmental conditions required to grow the cover crop of choice, the selection criteria must also include ease of establishment, effective absorption of excess fertilizer N, reduction of nutrient loss by leaching, and subsequent release of N for growth of a following crop (Isse et al., 1999). At the same time, NT and cover crops may exacerbate corn worm and borer infestations (Shapiro, 1999; Manley et al., 2002**)**, i.e.: armyworm (*Spodoptera frugiperda*), cutworm (*Agrotis ipsilon),* budworm and rootworms (*Diabrotica spp.*), earworm (*Helicoverpa zea*), European corn borers (*Ostrinia nubilalis*), particularly in those areas where these pests are a reoccurring problem. Since Bt-corn is resistant to these pests it might be the best hybrid choice for no-till/cover crop systems, increasing the acreage planted to Bt-corn and the importance of knowing the effects of Bt-corn residues on soil C and N dynamics.

Fertilizer

Comprehensive assessment of the total greenhouse gas (GHG) budget of agricultural systems must consider emissions of N_2O, NO_2 and CH_4 (Drury et al., 2006). It has been estimated that N fertilizer accounts for one-third of the GHGs produced by agriculture (IPCC, 2007), and according to USEIA calculations, the N fixation by leguminous crops planted worldwide adds as much N to the soil as commercial fertilizer; thus, theoretically, they would also contribute to N emissions (USEIA, 2007).

Optimum N inputs are essential for primary plant productivity, SOM stabilization, and SOC storage. The fertilizer efficacy depends on many variables including N source, application type, rate, timing and placement, and crop and residue management. As an example, N_2O emissions from corn crops grown on Canadian clay loam soils were not affected by any of the different tillage treatments when N-fertilizer was applied at a shallow depth but were influenced by tillage types when N was placed deep into soil. Furthermore, zone tillage reduced emissions relative to conventional and zero tillage. Thus, the authors concluded that zone tillage combined with shallow N placement were helpful practices to reduce N_2O emissions from corn crops grown on fine-textured soils in cool, humid climates (Drury et al., 2006).

Climate, soil moisture at the time of application, possible rainfall after application, soil structure, crop density, overall nutrient balance, soluble soil C availability and N conversion inhibitors (urease, nitrification) should be taken into account when choosing the N-fertilizer (Fontanetto et al., 2002). Excess N from both, inorganic (fertilizer) and organic fertilizers (manure, cover crops, etc.), is a potential source for NO_3^- leaching, and nitrogenous gas emissions from denitrification and volatilization processes. Organic sources release available N at a slower rate than inorganic compounds and may continue to release NO_3^- after crop uptake of N has ceased. Excess NO_3^- will be a source of leaching and denitrification derived emissions if conditions such as water filled pore space, temperature, and soluble C availability are dominant in the soil. Leaching is also affected by soil texture, fertilizer quantity, quality and application depth and time, soil moisture at the time of fertilizer application and abundant rainfall after application, as it may cause runoff losses of applied N.

When the soil holds the right balance of nutrients for optimum crop growth, the efficiency of applied and residual soil N are maximized. On the other hand, in accordance with Augustus von Liebig's “Law of the Minimum”, crop yield is proportional to the amount of the most limiting nutrient. Thus, any deficient nutrient that limits the plants’ development will affect N-uptake by limiting the plants’ growth; the “optimum” amount of applied N will then be in excess of the crop’s requirements and will be subject to more or less important N-losses depending on weather occurrence, cropping system and site-specific soil characteristics. However, increases in N fertilizer use do not always increase net CO_2 emissions. According to the last IPPC report (2007), intensive production systems that apply higher rates of N may have lower net global warming potential per unit of food production than low-input and organic production systems. Moreover, when the main objective is to obtain the higher possible yield, N inputs need to be high, especially in corn crops. However, higher corn yields do not necessarily mean higher farmer's profits; the higher N dose that will result in the higher grain yield will often give lower fertilizer efficiency. As an example, the efficiency of N applied with respect to corn yield or partial productivity factor (PPF) in the US Corn Belt is 58.4 for an average N application rate of 157 kg N per hectare, and an average yield of 9.9 metric tons corn per hectare. Argentina PPF averages 123.4 kg grain per kg N applied for an average of 58.1 kg N per hectare, and an average yield of 7.5 metric tons corn per hectare (Melgar, 2006).

Higher N losses often originate from soluble fertilizers, but many products have been developed to minimize potential N emissions to the atmosphere, including slow-release, controlled-release and stabilized N fertilizers. These kinds of fertilizers usually contain urease or nitrification inhibitors to enhance soil retention and crop uptake, and may reduce fertilizer-related short-term emissions depending on type and time of the application. For example, urea application to corn at the 6-leaf stage increased soil mineral N content at flowering, maize N uptake and grain yield resulting in better crop performance and fertilizer use efficiency than using urease inhibitor (Sainz-Rozas et al., 2001). The interaction of fertilizer type, depth, time and type of application with tillage type will further affect N_2O emissions. Researchers have found that emissions were higher under NT than under conservation tillage when urea was pre-plant broadcasted on maize crops, and the opposite happened when anhydrous NH_3 was soil injected (Sainz-Rozas et al., 2001; Vetsch and Randall, 2004). As already mentioned, the choice of crop, residue, and nutrient management are site-specific and have more than one solution. For example, the pre-plant broadcasting of urea-based fertilizers to NT corn requires the incorporation of urease inhibitors to prevent N losses, particularly in humid regions (Vetsch and Randall, 2004). Instead, many corn producers choose to apply N fertilizer twice, once at planting and once when the plants are at the 6-8 leaf stage to obtain similar results (Drury et al., 2006).

BIOFUELS

Substituting biofuels for gasoline or diesel saves non-renewable resources and reduces GHG emissions that impact global warming. Hundreds of millions of tons of corn biomass left on corn fields every year make stover an attractive feedstock for bioethanol production, and might increase the farmer's income provided that the gross returns of harvesting stover for biofuel production exceeds the cost of any additional nutrient that might be needed to compensate for the nutrients that will not be released in soils through stover decomposition. Planting cover crops might counteract the possible decrease in SOC and nutrients, and the potential increase of soil acidification due to the crop absorption of soil N and P if these are not returned with stover (USEIA, 2007). Since cover crops increase the risk of worm and borer infestations, the adoption of insect-resistant Bt-corn hybrids might be necessary, increasing the already large area of land planted to Bt-modified maize.

It has been proposed (Masoero et al., 1999; Saxena and Stotzky, 2001; Hopkins and Gregorich, 2003; Flores et al., 2005; Fang et al., 2007) that Bt-modified corn hybrids may have an increased amount of lignin that could affect its degradation in soil, the length of time required to release nutrients into the soil, and ethanol yield. Many corn hybrids such as the high fermentable corn (HFC) varieties have already been developed to produce a higher amount of biomass with increased degradability that promote higher ethanol yields and have other stacked traits including Bt-resistance to insects. Thus, the evaluation of possible variations in the chemical composition of stover of non-Bt and Bt-modified corn hybrids of HFC and non-HFC varieties and its possible prolonged biomass turnover become compelling issues to assess soil C and nutrients budgets.

Biofuels are renewable energy sources produced from plant biomass (bioethanol), vegetable oils and animal fat (biodiesel), and municipal and industrial wastes (biogas) in lieu of fossil fuels that are irreversibly exhausted once they have been used to produce energy. Ethanol is used instead of gasoline (Brazil) or as an octane booster, gasoline stock extender and a pollution-reducing additive for gasoline (USA) (USEIA, 2007). The major feedstock for ethanol in Brazil is sugar cane, while corn grain is the main raw material for ethanol in the US (Dien et al., 2002). In the United States, bioethanol is promoted as a way to reduce the dependence on foreign petroleum while reducing air pollution, water pollution, and GHG emissions (USEIA, 2007). In this connection, the US government has issued several federal policies that boosted biofuel related activities, which have been steadily growing since the mid 1990’s (USEIA, 2007). Overall, ethanol is expected to grow from 5.6 billion gallons in 2006 to 24.3 billion gallons by the year 2030 (> 16% of US total gasoline consumption volume), or about a 4 billion gallon increase each year (USEIA, 2007). Currently, most of the US production of ethanol comes from corn grain that requires high energy inputs during the conversion of grain starch into sugars, resulting in a poor net energy balance (energy liberated by ethanol minus energy to produce ethanol) of 25,000 BTU/gal. On the other hand, cellulose-based ethanol is produced mainly from corn residues or other cellulosic biomass, and utilizes a by-product of cellulose conversion into sugars as the heat source for the next conversion sequence, resulting in a much better net energy balance of 60,000 Btu/gal (USEIA, 2007). Thus, although grain-based corn ethanol still decreases foreign petroleum dependence, if the energy input comes from natural gas or electricity, cellulose-based corn ethanol seems to be a much better option. Moreover, in addition to the primary output of ethanol, the wet milling process generates corn gluten feed and meals that are converted into livestock feed, and corn oil that is sold for human consumption at a price higher than soybean oil. The by-products of dry mill ethanol are coarse, unfermented grains and a liquid fraction known as thin stillage, which are further processed into “distillers grains (DG)”. There is almost no starch in the DGs, consequently its content of essential nutrients including protein, fat, fiber, minerals, and vitamins, is three times higher than that of corn grain (USEIA, 2007) which makes it very useful for the food and feed industry.

Observing the potential highlighted importance of stover, seed companies seized the opportunity by developing corn hybrids that produce more stover (Monsanto’s Producer Preferred) (Monsanto, 2008a) and can deliver higher levels of fermentable starch (Monsanto’s High Fermentable Corn (HFC)) that allowed dry-mill plants to increase their ethanol output by 3-5% (Agbios, 2008; Monsanto, 2008b). Syngenta has recently developed hybrids that contain amylase to increase wet-mill grain-based ethanol efficiency (scheduled to be launched in 2008) (Agbios, 2008). The acreage of corn planted has escalated with the increase in ethanol production. Between 2006 and 2007, there was a 32% increase in US ethanol production that was mirrored by a rise in the acreage planted to corn (for all purposes) from 78.3 million acres in 2006 to 93.6 millions in 2007 (NASS, 2006a), 50% of which contained the Bt-protein (NASS, 2006b).

In the US Corn Belt, maize yields are highly dependent on N inputs. Therefore, farmers apply large amounts of N fertilizers that may not be completely removed from the soil with the harvest, leaving excess N in the soil that may be prone to loss through N_2O emission and NO_3^- leaching, increasing the risk of eutrophication in the Gulf of Mexico where an area of low dissolved O_2 (hypoxia) forms every summer (NSTCC, 2000). It has been calculated that between 1980 and 2000, the Mississippi and Atchafalaya Rivers discharged, on average, about 1.6 million metric tons (3.5 billion pounds) per year of total N to the Gulf of Mexico. Approximately 56 % is from the Mississippi River above the Ohio River and 34% from the Ohio Basin (NSTCC, 2000), where the agricultural land that produces more than 80% of US corn is located. The concentration of N in these rivers has been continually increasing, and by 2006 it was responsible for a hypoxic zone of 17,500 square kilometers (4.3 M acres) (Donner et al., 2008). The increase in corn cultivation required to meet the 15 to 36 billion gallons of bioethanol production by 2022 suggested by US policies (USEIA, 2007) could increase by 10 to 34% the average flux of dissolved inorganic N per year delivered by the Mississippi and Atchafalaya Rivers into the Gulf of Mexico (Donner et al., 2008).

The impact of both grain-based and cellulose-based corn ethanol on environmental pollution and CO_2 emission is still the object of many debates, however, its favorable effects on the economy of US rural communities, particularly those located in the Corn Belt, cannot be denied. The raw material used to produce approximately 90% of fuel ethanol comes mostly from the mid-western states located in the Corn Belt. Approximately 31.5 billion kilograms of corn, equal to 11% of the total U.S. corn crop, were processed to ethanol in 2004 (Nichols et al., 2005). Because of this, stover management has an increased importance for the farmers of this region. The knowledge about how much residue should be left on the field for erosion control, how much should be incorporated to maintain soil fertility, and how much might be sent to ethanol production is critical since the industry needs the commitment of the farmer to keep up with production.

It has been argued that the use of bioethanol is a significant improvement, in terms of environmental benefits, over traditional fossil fuels (Shapouri et al., 2004; Kim and Dale, 2005). This concept was reinforced by Sartori et al. (2006), who concluded that US bioenergy production had a “realistic potential capacity” to offset almost 20% of CO_2 emissions derived from fossil fuel consumption. However, Searchinger et al. (2008) stated that corn grain ethanol would not offset fossil fuel emissions, but would instead double CO_2 emissions over a 30-year period. Crutzen et al. (2008) affirmed that although the use of biofuels instead of petroleum-derived fuels could decrease CO_2 emissions, the same or a larger amount of CO_2 equivalents would be released in the form of N_2O. The conclusions of the various researchers may be combined but not compared because their predictions were made using different sets of variables and different computer models to simulate the interactions of the variables considered. Therefore, the data currently available are not sufficient to assess whether grain or lignocellulosic ethanol is better or worse than fossil fuel concerning GHG emissions, and further research is needed (Reijnders and Huijbregts, 2007). However, due to the large number of dynamically interrelated factors involved, it may not be possible to infer a general solution on this matter as even the results of a careful and thorough evaluation of the parameters of a specific site may shift from positive to negative (and vice versa) due to unforeseeable changes in climatic or socio-economic conditions. Overall, it is to be expected that the development of new chemical and biochemical technologies, the improvement of yield and performance of ethanol manufacturing processes coupled with adequate soil, water and crop management practices will eventually lead to a profitable and sustainable industry with improved environmental impact.

TRANSGENIC CORN

As already explained in the previous section, the production of ethanol from lignocellulosic biomass has been promoted on the basis that crop residues such as corn stover offer an abundant, inexpensive, and renewable source of fermentable sugars (Vermerris et al., 2007), with the added advantage over grain ethanol of having higher positive net energy balance and beneficial impact on CO_2 emission (Shapouri et al., 2004; Pimentel and Patzek, 2005; Farrel et al., 2006). Although the accuracy of these statements is still largely debated (Shapouri et al., 2004; Kim and Dale, 2005; Sartori et al., 2006; Crutzen et al., 2008; Searchinger et al., 2008) and conclusions/predictions are thus far limited to each particular case, the ethanol plants that have been built during the last decade in the US have dry-mill (lignocellulose-based) technology. Dry-mill corn ethanol production used less than 3.8 billion kilograms in 1996, but nearly 25 billion kilograms in the 2005-2006 crop year, while wet-mill corn ethanol has been relatively steady using just less than 12.5 billion kilograms per year during the same period of time (USEIA, 2007). This is in spite of the fact that about 80% of US ethanol facilities use wet mills (USEIA, 2007) and that most US corn grown for food, feed, and biofuel alike are still focused on high grain yield (Vermerris et al., 2007).

Matching this trend, seed companies have been breeding new corn hybrids that grow more foliage and engineering cultivars with altered starch properties to better suit the growing biofuel industry. They have developed a wide range of corn varieties combining transgenic technology and traditional breeding. Farmers currently have an ever-increasing spectrum of choices including herbicide-, insect- and disease-resistant hybrids; cultivars with increased biomass/grain yield and modified starch quality and quantity to increase the conversion efficiency for both wet and dry mill ethanol-producing facilities; specialty corns such as waxy, high-amylose, high-amylopectin, high-oil and high-lysine corns with characteristics developed to suit a number of industries including textile, food additives, candies, cosmetics, pharmaceutical; etc. (Tomasik, 2003; Vermerris et al., 2007). The starch of high-amylopectin waxy corns contains almost 100% amylopectin for higher livestock/animal digestibility and increased ethanol output efficiency. Normal maize starch has a ratio of approximately 75% amylopectin to 25% amylose. Amylopectin is water-soluble and is easily digested by animals and humans alike, while ethanol conversion efficiency increases as the amylose content decreases, particularly when the amylose content is smaller than 35% (Wu et al., 2006). High-amylose waxy corns provide starch containing up to 90% amylose that is hard to digest but has improved hydrocolloid properties for gels, films, biodegradable polymers, etc. (Tomasik, 2003; Jaeger et al., 2006). “Leafy” corn hybrids produce up to twice as many leaves above the ear, and about 10-12% higher biomass yield than normal corn (Andrews et al., 2000). Dow Agrosciences claims that their fourth-generation brown mid-rib (BMR) hybrids yield on average two tons more per acre than previous generation hybrids, and have lower lignin content that increases fiber digestibility and dry matter intake in cattle, which in turn produces an average of 10.6 kilograms more milk per cow per day when fed with this corn silage (DOW, 2008).

These leafy hybrids have a smaller rind (outer portion of the stalk) than conventional corn. The rind is a major factor for maize plant stability and is very indigestible compared to the inner stalk, or pith, that is composed mostly of fiber and digestible nutrients. At the same time, the softer stalk and the increased size of these leafy hybrids increased the incidence of insect and disease attacks and its associated crop risks (Bullock and Nitsi, 2001). Thus, most of the corn varieties sown in the US have two or more “stacked” traits. According to the USDA (2008a), 73% of the US total corn (for all purposes) acreage of 2007 was planted with bioengineered hybrids; 49% of them contained the insect resistant (Bt) trait alone (21%) or stacked (28%) with other traits. For example, Monsanto’s HFC (high fermentable corn) 3410 is a tetraploid (contains twice the genetic material of a conventional diploid maize) that produces a higher grain and/or stover yield (Processor Preferred (PP) trait) than conventional corn (Monsanto, 2008a). The quality and enzymatic composition of the starch in its grain have been modified to give a higher ethanol conversion (high total fermentable starch (HTF) trait). It also contains an insecticide Bt-protein that confers resistance to European corn borer attacks (YG-trait) plus an enzyme that makes it resistant to glyphosate herbicide (RR-trait) (Seeds, 2008). When this HFC corn is processed through a dry grind mill, the ethanol output increases by up to 5%. That represents an increase in profitability of $2 to 5 million for 40 million gallons (151 million liters) per year for ethanol production (Monsanto, 2008b; Seeds, 2008).

The above-mentioned desirable qualities, as well as the enticing characteristics of many other corn varieties on the market, also produce a cascade of interrelated dynamic effects on the biosphere. This must be added to the already long list of variables (tillage, cover crop, crop rotation, crop density, nutrient and water management and availability, soil physical and chemical properties, etc.) that need to be considered when deciding the amount of corn stover that can be removed from every specific site as modified by the prevalent climate conditions of each season. Furthermore, each one of the variables modifies and is modified by the rate of above and below ground corn stover degradation that in turn impacts soil fertility, agricultural productivity, and soil environmental conditions. Thus, the importance of increasing our knowledge about corn stover decomposition in soils, which is the main objective of this study, can hardly be overemphasized.

CHAPTER III

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Two parallel studies were carried out. The first study was designed to compare the effects of location, Bt protein (genotype), plant portions, and their interactions on the total concentration of N and carbon in soils amended with stover of DKC69-71(Bt) and DKC69-72(non-Bt) plants (grown at two different locations) over a 223-day incubation period.

The second study was designed to compare the effects of genotype and plant portions (between-subjects factors) and their interactions on the total concentration of N and carbon in soils amended with stover derived from three portions (upper, middle and lower) of Bt/non-Bt corn (non-HFC) hybrids and stover derived from Bt/non-Bt HFC hybrids grown under identical circumstances. The location factor was not included in this design in order to highlight the differences caused by the HFC trait. Samples were incubated and analyzed simultaneously with those of the first experiment during the same 223-day incubation period.

The experimental design of the first experiment was a full factorial design with four replications that included the factors of location, genotype and plant-portion (upper, middle and lower) for corn hybrids DKC69-71(Bt) and DKC69-72(non-Bt) over time (223-day period). The experimental design of the second experiment was a full factorial design with four replications, including the factors of genotype and plant-portions (upper, middle and lower) of the corn hybrids DKC69-71(Bt), DKC69-72(non-Bt), HFC DKC34-11(Bt), and HFC DKC34-10(non-Bt), over a 223-day period of time.

The full factorial design was chosen because it allowed the examination of the individual or “main” effect of each factor on carbon and N mineralization, and had the advantage over other designs of showing the interaction effects that existed between factors. Furthermore, although interactions occur between factors, the factorial design showed if an interaction effect existed when differences in one factor such as genotype depended on the level (location) of another factor (Zolman, 1993).

SOIL SAMPLE

A bulk soil sample was collected at the Texas A&M University Agricultural Experiment Station Research Farm (30˚ 32′ N, 96˚ 26′ W) in Burleson County near College Station, Texas, from a site that had not been cropped during the last 20 years. The soil was a Weswood silty clay loam (fine-silty, mixed, superactive, thermic Udifluventic Haplustepts) (NRCS, 2002) with 181 g sand kg^{-1} soil, 486 g silt kg^{-1} soil, 333 g clay kg^{-1} soil, 1.4 g cm^{-3} bulk density, pH 8.1, 32 mg extractable NO_3^--N g^{-1} soil, 8 mg extractable NH_4^+-N kg^{-1} soil and, 16.1 g total C kg^{-1} soil, and 92 g $CaCO_3$ kg^{-1} soil. The soil was oven dried at 55 ˚C and ground to pass a 2-mm sieve before use in the experiments.

STOVER COLLECTION

Corn stover of DKC69-71(Bt) and DKC69-72 (non-Bt) hybrids were collected at harvest from the Texas A&M University Farm near College Station, Texas and Monsanto Experimental plots near Snook, Texas (30˚ 33’N, 96˚ 27’W). High-fermentable corn (HFC) varieties DKC34-11(Bt) and DKC34-10(non-Bt) were collected at harvest from Snook only. Both locations were yield-trial plots with randomized complete block designs with four replications. At College Station, corn was planted on March 11, 2004 on Ships clay loam soil (very fine, mixed, thermic Udic Chromustert) and stover was harvested on July 17, 2004. The purpose of the test at this location was to determine the yields of corn hybrids under conditions representative of the Brazos Bottom near College Station. Due to the particular weather conditions of that year, the researchers decided not to irrigate. Rainfall occurred during the tassel-silk stage and during grain fill and maturation stages. According to the researchers *(Dr. F.J. Betran Associate Professor, Soil & Crop Dept., Texas A&M University, personal communication),* the test suffered a short period of drought stress just prior to flowering.

Although primarily focused on yield performance, the objective of the research was to find the most suitable hybrids to grow in the river basin with site-specific irrigation management and water use since optimizing water utilization is a major factor in successfully growing corn in Texas. During the first half of the year 2004, this area of Texas received the equivalent of the normal annual rainfall. In many locations, June precipitation was either the highest or the second highest ever recorded (National Weather Service, 2005). Therefore, researchers decided not to irrigate their plots at College Station. In fact, Texas climate statistics indicated that during 2004, temperatures (Fig. 1) and rainfall (Fig. 2) were higher than the normal average for College Station and surrounding areas.

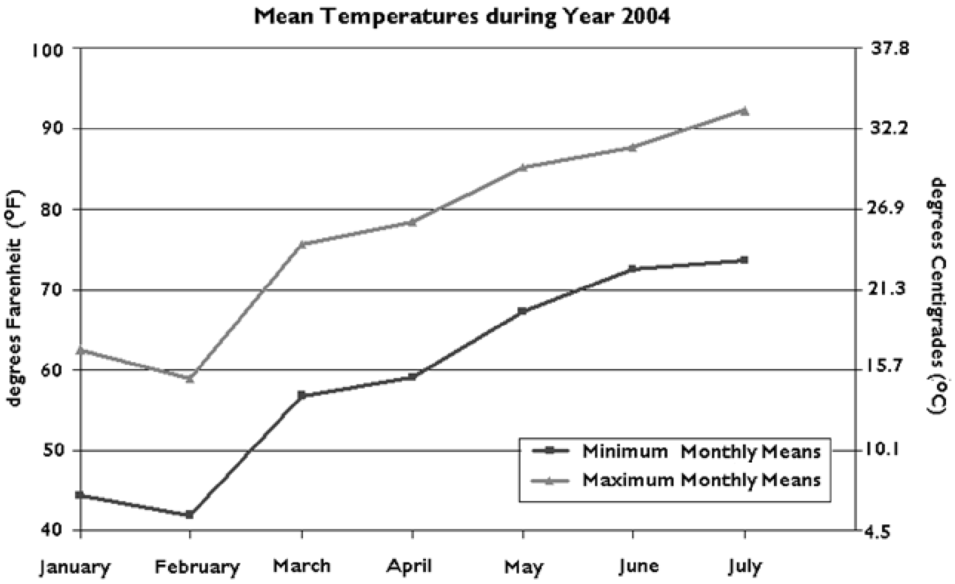

Fig. 1. Mean temperatures during 2004 corn growing season in College Station, TX.

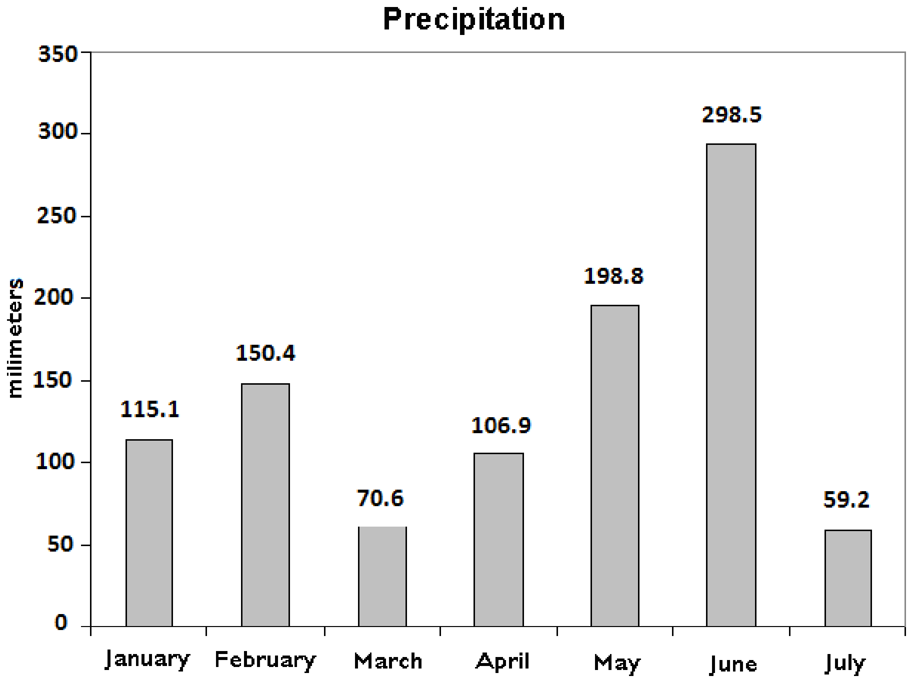

Fig. 2. Monthly rain in millimeters during the first half of 2004 in College Station, TX.

At Snook, corn was planted on March 9, 2004 on Zulch sandy loam soil (fine-loamy, smectitic, thermic, Udertic Paleustalfs) and stover was harvested on July 9, 2004. The plants in this plot were grown under irrigation.

STOVER SAMPLES PREPARATION

Aboveground tissues (no ears) from three plants per row were harvested at maturity. Each plant was divided into three portions: 1) lower - first 3 nodes, no roots, 2) middle - third to the next-to-last node, and 3) upper - next to the last node to the top of the plant, including the tassels. The lower and middle parts had about the same amount of leaves, the upper portion had relatively more leaf mass because the stalks in this part of plant were thinner. Each portion was placed into paper bags labeled to identify location, plant row, and plant portion. At the end of harvest, all paper bags were placed into a forced-draft oven, at approximately 50 ˚C for 24 hours. Stover was then ground in a Wiley mill to pass through a 2-mm mesh sieve. The material from plants of the same row and location were pooled (keeping plant portions separate) and stored in tightly sealed plastic bags at room temperature.

Isolines grown at both locations included DKC69-71 (RR2/YGCB), Residue Proven (119 day maturity), and DKC69-72 RR2, Residue Proven (119 day maturity). Isolines growing only near Snook, Texas, included DKC34-10 (RR2), HFC, Residue Proven (114 day maturity), and DKC34-11 (RR2/YGCB), HFC, Residue Proven (114 day maturity).

The term RR2 (Roundup Ready 2) mentioned above refers to the hybrid’s resistance to the herbicide glyphosate provided by event NK603 through the introduction, by particle bombardment, of the modified 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimate biochemical pathway for the production of aromatic amino acids. The term YGCB (Yield Guard Corn Borer) refers to the hybrid's resistance to corn borer [*Ostrinia nubialis* (Hubner)] conferred by event MON810 which is the insertion of Cry1Ab genes from *Bacillus thuringiensis* subspecies *Kurstaki* (Btk) by microparticle bombardment of plant cells or tissue. The term HFC (high fermentable corn) refers to corn varieties that can deliver higher levels of fermentable starch to dry mill ethanol plants. According to the seed company, this hybrid may increase ethanol output by 3-5% (Monsanto, 2008b). Finally, the term Residue Proven refers to corn varieties developed to produce high yields of stover in reduced tillage systems (Monsanto, 2008a).

Forty-gram (oven-dry basis) soil samples were used for each replicate and were added to 50-mL beakers. Two-hundred (200) milligrams of ground stover were added to each 40-g soil sample. The quantity of stover added was based on the average yield of DKC69-71 (~200 bushel/acre) attained in the 2003 College Station Corn Performance Test, Texas A&M University Farm, College Station, Texas.

TISSUE ANALYSIS

The N and C concentration of stover samples was analyzed by the AgriLife Extension Soil, Water and Forage Testing Laboratory at the Soil and Crop Sciences Department of Texas A&M University (College Station, TX) with a combustion gas analyzer following Method 972.43 of Official Methods of Analysis of AOAC International, 1997, using a dynamic flash combustion system coupled with gas chromatographic (GC) separation system and thermal conductivity detection (TCD) system. The method (AOAC, 1997) had a detection limit of 0.01% for C and 0.04% for N (generally reproducible within ± 5% for C and ± 7% for N).

Basic analysis of stover composition was performed according to methods of Van Soest et al. (1991). Starch content was analyzed with a Sigma SA-20 Starch Assay Kit following the manufacturer's instructions.

LABORATORY INCUBATION STUDY

Carbon Mineralization

Ground stover was thoroughly mixed with the soil, and N in aqueous solution as NH_4NO_3 was added to the blanks (40 g soil; 50 mg N/kg soil) and samples (40 g soil + 200 mg ground corn stover), together with 11 mL of de-ionized water. This was done to approximate the C:N ratio of the soil/stover mixture to about 30:1, with a soil moisture content of approximately 50% of the soil water-holding capacity. Temperature was kept constant at 24 ± 2 ˚C throughout incubation. According to Recous et al. (1995), these conditions should eliminate most other variables to reveal the effect of Bt modification on stover decomposition rate as measured by C mineralization.

Decomposition rate was determined by measuring the amount of C respired as CO_2 and absorbed into an alkaline solution over time. Four replications per sample and four replicated controls without plant residues were incubated in sealed glass jars containing a trapping solution vial with 10 mL 1N KOH, and a vial containing 15 mL of de-ionized water to insure sufficient moisture levels during the experiments. Trapping solutions were titrated at days 1, 2, 3, 7, 14, 21, 35, 57, 95 and 223 of incubation, with traps being replaced with fresh alkali following each titration. The CO_2-C captured by the 1N KOH solution was determined by titrating with 1N HCl to a phenolphthalein endpoint after the precipitation of the carbonates with excess 3M $BaCl_2$ (MacFadyen, 1970; Zibilske, 1994).

The amount of C mineralized was calculated as:

$$\text{mg } CO_2 = (B \text{ mL} - V \text{ mL})*N*E$$

where

B = volume of standard HCl consumed in the titration of blanks to the endpoint

V = volume of HCl consumed to titrate the trap solution from the sample jars to the endpoint

N = normality of HCl

E = 22 equivalent weight of CO_2

The figures thus obtained represented CO_2 mineralized by 40 g soil samples and were multiplied by 25 to obtain mg CO_2 per kg soil.

Nitrogen Mineralization

At the end of the 223-day incubation period, soil samples were oven dried at ~ 40 °C, and then pulverized to pass a 60-mesh sieve. The total C and N concentrations of samples were analyzed by the Soil, Water and Forage Testing Laboratory at the Soil and Crop Sciences Department of Texas A&M University (College Station, TX) using a combustion gas analyzer following Method 972.43 of Official Methods of Analysis of AOAC International (1997). This quantitatively determined the total amount of N and C in all forms in soil, botanical, and miscellaneous materials by using a dynamic flash combustion system coupled with a gas chromatographic (GC) separation system and thermal conductivity detection (TCD) system. The analytical method was based on the complete and instantaneous oxidation of the sample by flash combustion, which converts all organic and inorganic substances into combustion gases (N_2, NO_x, CO_2, and H_2O). This method (AOAC, 1997) had a detection limit of 0.01% for C and 0.04% for N (generally reproducible within ± 5% for C and ± 7% for N). Total soil N was also determined after Kjeldahl digestion of 1.000 ± 0.005 g of oven-dry soil samples ground to pass a 10-mesh sieve (< 2.0 mm). This digestion procedure did not quantitatively digest N from heterocyclic compounds, nitrates, or ammonium from within mineral lattice structures. The procedure had a detection limit of approximately 0.020% N and is generally reproducible within ± 8% (Bremner and Mulvaney, 1982). Nitrate-N (NO_3-N) in soils was determined spectrophotometrically following extraction with 1N KCl.

The effect of adding residues on the dynamics of soil mineral N (net mineralization or immobilization) was calculated from the difference between the mineral N present in soil + residue mixture and that present in the controls (soil, no stover added, incubated under identical conditions). The amount of N released was compared with the rate of C lost as CO_2.

STATISTICAL ANALYSES

The data were analyzed using several test options of the SPSS statistical software (SPSS, 2002). The repeated-measures ANOVA test was used because a standard ANOVA would not model the correlation between the repeated-measures (10 measurements made on each sample between day 1 and day 223) made over time and, therefore, the data would violate the ANOVA test assumption of independence.

Next, a general linear model (GLM) was run because this program can identify the effects involving between-group and repeated-measures factors, and can plot the results in a wide variety of ways including the interactions with their corresponding standard error bars. GLM also provided a validation of the ANOVA test.

Finally, a general regression model (GRM) was run. The GRM stepwise and best subset tests were then used to quantify the main effects and the percentage contribution of each factor/level combination and interaction effects, to the differences observed in C and N mineralization. This test also provided a validation of the results obtained in the GLM test for the between groups and repeated measures effects.

In all statistical methods for the analysis of experimental data, the results will have statistical significance if enough samples are analyzed. The combined results of the three tests described above provided a way to determine if the effects that were statistically significant were also agronomically significant, or if they had to be considered within the range of normal variations that occur when corn plants are cultivated under field conditions.

In all comparisons, $\alpha = 0.05$ was used to protect against type I errors. Contrast procedures were performed to verify differences among the various combinations location*genotype*plant portion or genotype*plant for the high fermentable corn hybrids. Regression processes were used to determine the effects/relationship of location, genotype, plant portions (or genotype and plant portion), and stover components on the C and N content of soil. Pearson’s two-tail correlation was used to observe the size and sign of the interactions between structural and non-structural components and N content of stover samples with mineralization parameters.

CHAPTER IV

RESULTS AND DISCUSSION

This study was designed to investigate the chemical composition (starch, cellulose, hemicelluloses and lignin) and the total N concentration of upper, middle and lower portions of two Bt and non-Bt isogenic hybrids of corn (*Zea mays* L.), namely, DKC69-71Bt/ DKC69-72(non-Bt) and the high fermentable corn variety DKC34-11Bt/ DKC34-10(non-Bt), and their effects on the C and N mineralization of stover incorporated into a calcareous Weswood soil.

Previous research studies performed under laboratory conditions indicated that transgenic crops expressing the Cry1Ab insecticidal protein from *Bacillus thuringiensis* (Bt) decomposed at a slower rate (as determined by CO_2 evolution) than their respective non-Bt isolines (Dinel et al., 2003; Flores et al., 2005). Field studies also showed a lower CO_2 evolution from soils under Bt corn cropping or amended with Bt corn biomass than from soils under non-Bt maize culture or amended with non-Bt corn hybrids (Dinel et al., 2003; Castaldini et al., 2005). On the other hand, some researchers (Hopkins and Gregorich, 2003; Lehman et al., 2008) did not observe any differences in the degradation of Bt and non-Bt corn, and found no direct effects of the purified Bt toxin or the Bt toxin contained in the plant tissue on the decomposition of maize residues in soil (Baumgarte and Tebbe, 2005).

Changes in soil decomposition of stover from Bt-modified corn compared with non-Bt modified corn may be affected by and may affect many variables including:

a) Management practices as they affect biotic and abiotic factors that influence residue degradation and nutrient turnover.

b) Alteration in the composition and physical form of the maize hybrids (transgenic and non-transgenic) caused by environmental conditions such as soil characteristics, water and nutrient availability, etc., prevailing during crop development.

c) Modification of the composition and/or other traits of the transgenic plant as a result of unintended and unexpected secondary effects of Bt-gene insertion (pleiotropy).

d) Inhibition or stimulation of soil microbial communities involved in the residue transformations in soil.

The present experiment was conducted in the laboratory with soil that had not been cropped for over 20 years, under controlled humidity and temperature, and non-limiting N conditions. All stover was oven dried, ground and incorporated into the soil to maximize the contact between stover and soil. Thus, most of the variables mentioned under (a) above were eliminated.

RESIDUE DECOMPOSITION (CO_2 EVOLUTION)

The results of my experiments showed that at the beginning of the incubation, C was mineralized at a slower rate in the soil samples amended with stover derived from the DKC69-71 Bt-modified corn plants than in soil samples receiving stover of the corresponding isogenic non-Bt hybrids (Figures 3 and 4). The differences were more pronounced for corn grown at the Snook, TX location. The differences between the degradation rates tended to decrease or disappear as time elapsed.

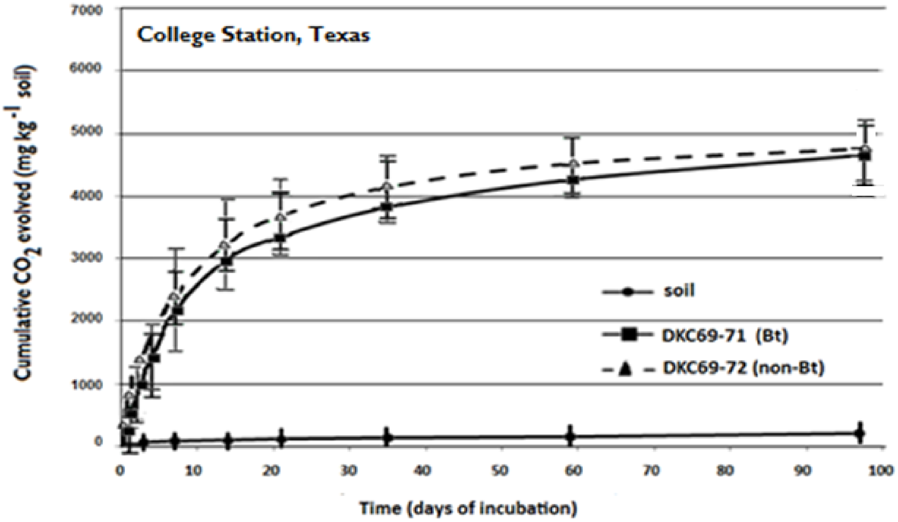

Fig. 3. Means of cumulative CO_2 (mg kg^{-1} soil) evolved from soil samples amended with stover of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station, TX. Curves represent the total mean for the three plant portions. Error bars represent ± SD.

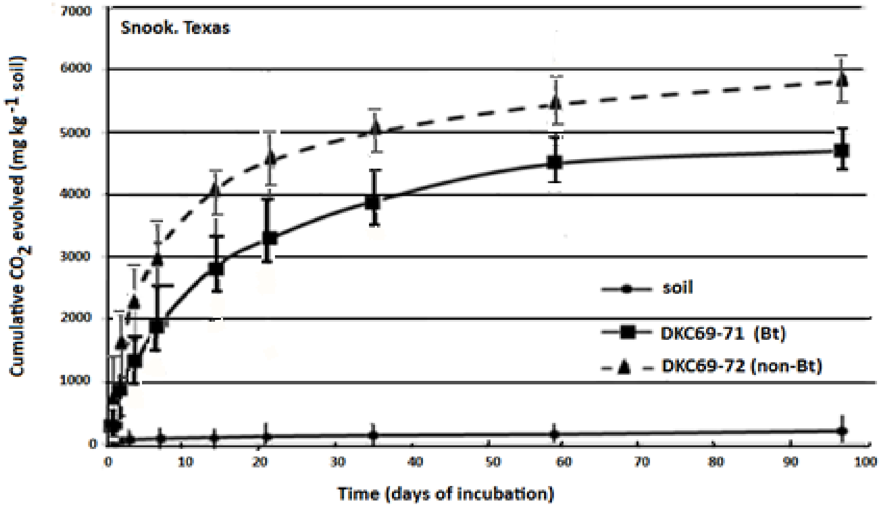

Fig. 4. Means of cumulative CO_2 (mg kg^{-1} soil) evolved from soil samples amended with stover of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at Snook, TX. Curves represent the total mean for the three plant portions. Error bars indicate ± SD.

The goodness of fit to the exponential first-order kinetics curve model used to calculate the mineralization rate (k day^{-1}) was excellent at days 7, 14 and 21 of incubation, slightly less at day 35, and still fairly good at day 223 (Appendix, Table A-1). There were no significant differences between the degradation rates of stovers of the non-Bt and Bt-modified lines of the high-fermentable corn (HFC) variety grown at Snook (Figure 5, Appendix Table A-1), although there was a slight trend for greater degradation of the non-Bt line.

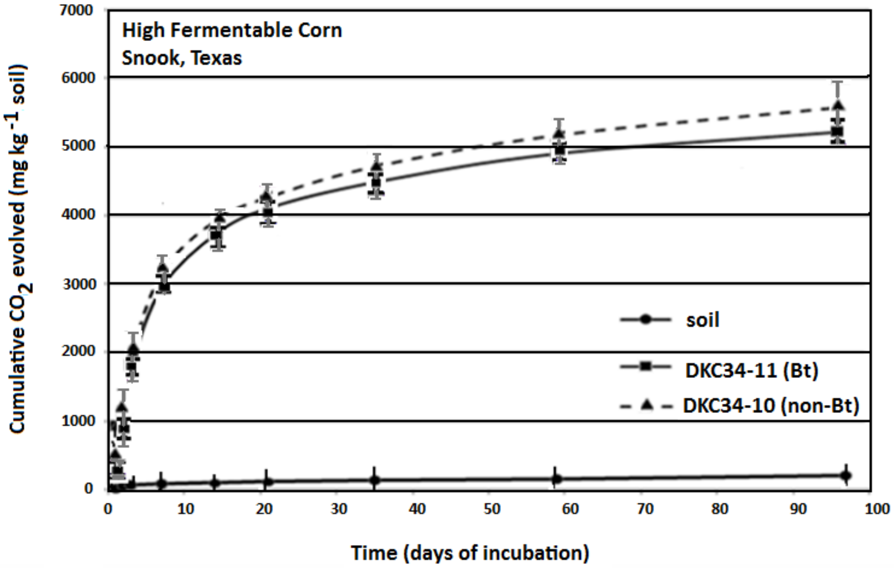

Fig. 5. Means of cumulative CO_2 (mg kg^{-1} soil) evolved from soil samples amended with stover of DKC34-11(Bt) and DKC34-10(non-Bt) corn hybrids grown at Snook, TX. Curves represent the total mean for the three plant portions. Error bars represent ± SD.

During the first day of incubation, soil samples containing stover of the non-Bt (non-HFC DKC69-72) plants evolved about 3X more CO_2 than the soil samples amended with stover of the Bt-counterpart grown at Snook, TX (Figure 6a, Table 1), and also about 3X more CO_2 than soil samples amended with stover of the same non-Bt hybrid grown at College Station (Figure 6b, Table 1). However, a similar amount of C was mineralized from stover of the Bt-modified plants of the non-HFC DKC69-71 variety, regardless of the location where the plants were grown (Figure 6a and 6b, Table 1). Furthermore, soil samples amended with the lower portion of the DKC69-72 (non-Bt) plants grown at College Station, TX respired approximately 3 fold more CO_2 than soil samples receiving either the upper or middle portions of the same plants (Figure 6b, Table 1), while about the same amount of CO_2 evolved from soils samples receiving stover of the various portions of this hybrid (non HFC DKC69-72(non-Bt) grown under irrigation at Snook, TX (Figure 6a, Table 1).

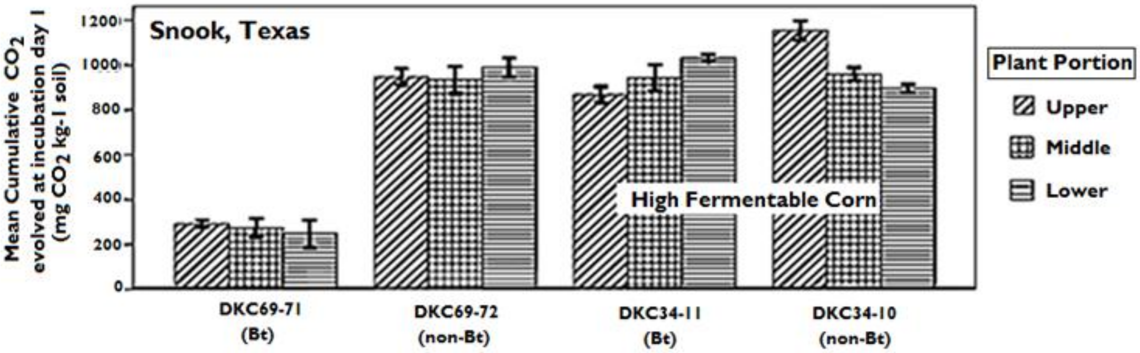

Fig. 6a. Means of cumulative CO_2 (mg kg^{-1} soil) evolved during day one of incubation from soil samples amended with stover of DKC69-71(Bt), DKC69-72(non-Bt), HFC DKC34-11(Bt) and DKC34-10(non-Bt) corn hybrids grown at Snook, TX. Error bars represent ± 2 SD.

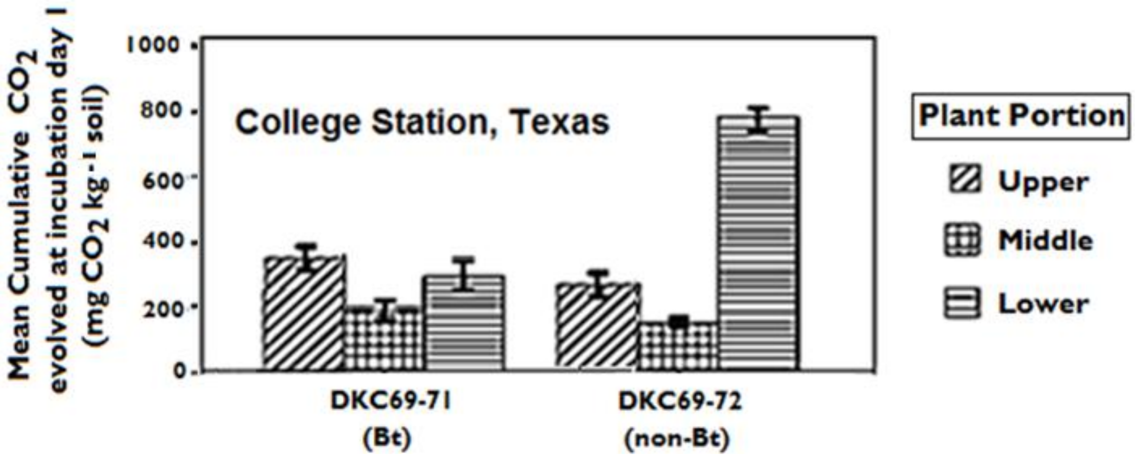

Fig. 6b. Means of cumulative CO_2 (mg kg^{-1} soil) evolved during day one of incubation from soil samples amended with stover of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station, TX. Error bars represent ± 2 SD.

Table 1. Means of CO_2 (mg kg^{-1} soil) evolved during the first day of incubation from soil samples amended with stover of the upper, middle and lower portions of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX.

		mg CO_2 evolved kg^{-1} soil	
Location	**Plant Portion**	**DKC69-71(Bt) Mean ± SD**	**DKC69-72(non-Bt) Mean ± SD**
College Station TX	Upper	209.05 ± 11.76 (a)	241.16 ± 13.18 (b)
	Middle	168.61 ± 10.43 (a)	138.07 ± 5.58 (a)
	Lower	262.24 ± 19.21 (a)	691.38 ± 13.92 (b)
Snook TX	Upper	291.71 ± 8.24 (a)	946.61 ± 18.06 (b)
	Middle	274.24 ± 20.68 (a)	933.48 ± 30.42 (b)
	Lower	247.65 ± 30.67 (a)	989.51 ± 20.66 (b)

- Each value is the mean of four replicated laboratory incubations of the same portion of 3 different corn plants from each location. The figures represent values of CO_2 minus the average of CO_2 evolved from 8 samples of unamended soil incubated during one day.
- Means in the same row followed by different letters are significantly different according to *post hoc* Tukey's HSD method (equal variances assumed) ($p<0.05$).
- Comparisons are made between the same plant portions of isogenic Bt vs. non-Bt corn hybrids grown at the same location.

There was a slight, though non-significant, trend for greater (5-9%) degradation of the middle and lower portions of the HFC Bt corn during the first day of incubation

(Table 2). A significantly higher (~18%) CO_2 evolution from soil samples containing the upper portion of the HFC non-Bt plants was observed.

Table 2. Means of CO_2 (mg kg^{-1} soil) evolved during the first day of incubation from soil samples amended with stover of the upper, middle and lower portions of high fermentable corn hybrids DKC34-11(Bt) and DKC34-10(non-Bt) grown at Snook, TX.

	mg CO_2 evolved kg^{-1} soil	
Plant Portion	**HFC DKC34-11(Bt) Mean ± SD**	**HFC DKC34-10(non-Bt) Mean ± SD**
Upper	849.72 ± 63.07 (a)	1093.32 ± 46.27 (b)
Middle	987.78 ± 89.99 (a)	942.07 ± 64.41 (a)
Lower	988.82 ± 45.79 (a)	902.20 ± 78.25 (a)

- Each value is the mean of four replicated laboratory incubations of the same portion of 3 different corn plants from each location. The figures represent values of CO_2 minus the average of CO_2 evolved from 8 samples of unamended soil incubated during one day.
- Means in the same row followed by different letters are significantly different according to *post hoc* Tukey's HSD method (equal variances assumed) ($p<0.05$).
- Comparisons are made between the same plant portions of isogenic HFC Bt vs. HFC non-Bt corn hybrids.

Water-stressed corn usually has higher sugar concentrations, less starch, higher crude protein, higher crude fiber and more digestible fiber compared to non-stressed corn (Thakur & Rai, 1980; Frederick et al., 1990). Insufficient water availability is also known to promote redistribution of starch along the plant (Casel & Vough, 2006), thus, it was likely that corn plants grown under different water regimes could have responded with changes in their structural composition that may have led to the differences in the CO_2 flux observed during the first day of incubation. Laboratory results showed that the overall concentration of starch in the whole plant was not affected but its partitioning in the three different plant portions appeared to be affected by water availability (see table on page 78), Bt-trait and variety (see tables on pages 78 and 79). Starch concentration did not appear to be the cause of the differences observed in CO_2 evolution suggesting a probably higher accumulation of soluble sugars in the non-HFC non-Bt hybrids, particularly when grown under irrigated conditions. The non-HFC corn plants were taken from experimental plots where this particular corn line was being tested for its suitability to the water-stressed conditions usually present during the south central Texas corn growing season. These hybrids were promoted for their high yield and stress tolerance. One of the characteristics of this corn line, commercially known as “stay green”, is a trait that confers drought tolerance, stalk strength and higher than normal levels of photosynthate in the stalks (Vermerris et al., 2007).

A number of requirements must be fulfilled before any particular hybrid line can express phenotypically the potential characteristics for which it has been bred, including the collaboration of many genes, certain environmental conditions, or the concomitant occurrence of both at a particular point in time of the plant development (Hodgkin, 1998; Klingenberg, 2005). Therefore, it is not unusual to find that two or more apparently unrelated genes interact in the expression of one or more traits, or that the expression of those traits are being turned on or off by environmental conditions (Hodgkin, 1998).

Thus, the observed differences in CO_2 evolution during the first day of incubation, suggested that the Bt-trait may have interacted with one or more traits of the noon-HFC corn line, causing possible changes in the chemical structure as the corn plants responded to the environmental conditions. If the degree of such changes was beyond the normal varietal and plant to plant differences usually encountered in corn stover, there could have been noticeable differences in the cumulative C mineralization at the end of the 223-day incubation period. However, although there seemed to be an overall tendency of the soil samples amended with stover of the Bt-modified plants of the two hybrid lines under study to evolve lower (about 15% less in the HFCs and 10% less in the non-HFC hybrids) cumulative amounts of CO_2 than soil samples containing the isogenic non-Bt-derived stovers (Figures 7a and 7b), the differences between the mean cumulative CO_2 evolved by day 223 of incubation ($^{223}CO_2$) were not statistically different (Tables 3 and 4) except between soil samples amended with Bt/non-Bt non-HFC plants grown at Snook, TX and the lower portion of the same non-HFC genotypic pair grown at College Station, TX (Table 3).

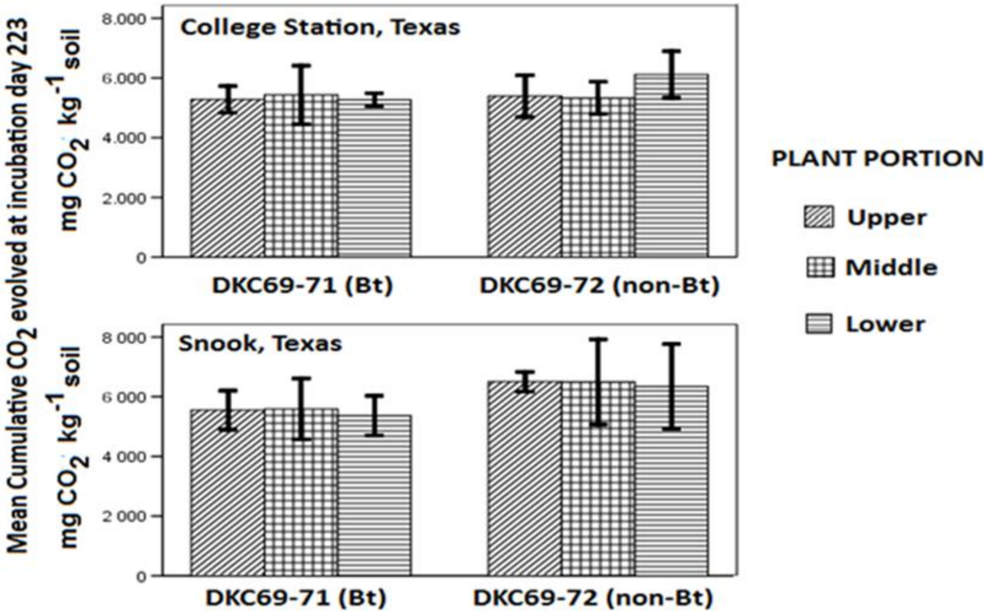

Fig. 7a. Means of cumulative CO_2 (mg kg^{-1} soil) evolved during 223 days of incubation from soil samples amended with stover of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX. Error bars represent ± 2 SD.

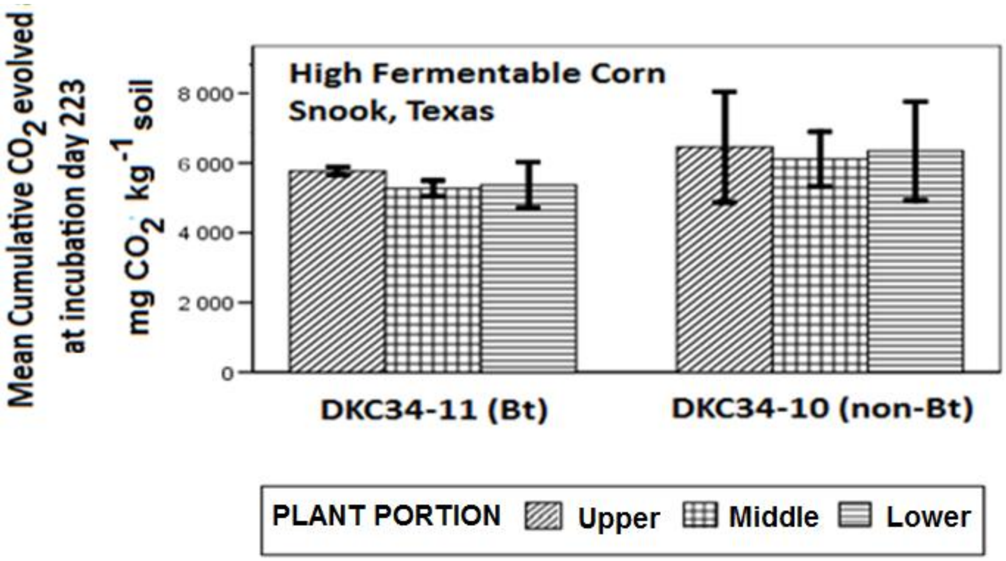

Fig. 7b. Means of cumulative CO_2 (mg kg^{-1} soil) evolved during 223 days of incubation from soil samples amended with stover of DKC34-11(Bt) and DKC34-10(non-Bt) corn hybrids grown at Snook, TX. Error bars represent ± 2 SD.

Table 3. Means of cumulative CO_2 (mg kg^{-1} soil) evolved during 223 days of incubation from soil samples amended with stover of the upper, middle and lower portions of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX.

		Cumulative CO_2 evolved during 223 days (mg kg^{-1} soil)	
Location	**Plant Portion**	**DKC69-71(Bt) Mean ± SD**	**DKC69-72(non-Bt) Mean ± SD**
College Station TX	Upper	5072.01 ± 223.85 (a)	5177.52 ± 346.54 (a)
	Middle	5220.17 ± 489.53 (a)	5119.54 ± 268.66 (a)
	Lower	5064.66 ± 107.54 (a)	5903.98 ± 388.99 (b)
Snook TX	Upper	5336.33 ± 327.44 (a)	6289.21 ± 167.20 (b)
	Middle	5380.56 ± 512.60 (a)	6286.69 ± 312.78 (b)
	Lower	5152.31 ± 330.55 (a)	6133.15 ± 708.91 (b)

- Each value is the mean of four replicated laboratory incubations of the same portion of 3 different corn plants from each location.
- Means in the same row followed by different letters are significantly different according to *post-hoc* Tamhane's method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between the same plant portions of isogenic Bt vs. non-Bt corn plants grown at the same location.

Table 4. Means of cumulative CO_2 (mg kg^{-1} soil) evolved during 223 days of incubation from soil samples amended with stover of the upper, middle and lower portions of high fermentable corn hybrids DKC34-11(Bt) and DKC34-10(non-Bt) grown at Snook, TX.

	Cumulative CO_2 evolved during 223 days (mg kg^{-1} soil)	
Plant Portion	**HFC DKC34-11(Bt) Mean ± SD**	**HFC DKC34-10(non-Bt) Mean ± SD**
Upper	5555.87 ± 61.02 (a)	6406.73 ± 324.32 (a)
Middle	5690.85 ± 237.06 (a)	6116.41 ± 252.34 (a)
Lower	5748.14 ± 241.24 (a)	6040.43 ± 303.85 (a)

- Each value is the mean of four replicated laboratory incubations of the same portion of 3 different corn plants.
- Means in the same row followed by different letters are significantly different according to *post-hoc* Tamhane's method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between the same plant portions of isogenic Bt vs. non-Bt HFC corn hybrids.

SOIL CARBON CONCENTRATION

At the end of the 223-day incubation period, there were no statistically significant differences in total soil C concentrations between soil samples amended with stover of the Bt vs. non-Bt corn plants of both the HFC and non-HFC corn hybrid lines (Tables 5 and 6) used in this study. No noticeable trend in soil C concentration was found to match the trend observed in the cumulative CO_2 evolution from the residue-amended soil samples (Tables 3 and 4). One possible explanation for the lack of correlation is that the small differences (<0.07%) observed between soil C concentration of samples receiving non-Bt or Bt-corn residues (Tables 5 and 6) could have been offset by equally small and mostly non-significant differences in stover C concentration (see tables on page 73).

Table 5. Means of soil total carbon (g C kg^{-1} soil) in samples amended with stover of the upper, middle and lower portions of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX after 223 days of incubation.

Location	Plant Portion	Soil Total Carbon* (g C kg^{-1} soil) DKC69-71(Bt) Mean ± SD	 DKC69-72(non-Bt) Mean ± SD
College Station TX	Upper	15.6 ± 0.1 (a)	15.3 ± 0.2 (a)
	Middle	15.0 ± 0.1 (a)	15.2 ± 0.0 (a)
	Lower	15.1 ± 0.0 (a)	15.3 ± 0.2 (a)
Snook TX	Upper	15.1 ± 0.1 (a)	15.5 ± 0.2 (a)
	Middle	15.3 ± 0.1 (a)	15.3 ± 0.1 (a)
	Lower	15.2 ± 0.1 (a)	15.3 ± 0.1 (a)

- (*) Difference between total soil C concentration of samples amended with stover and the averaged total soil C concentration of 8 unamended soil samples.
- Each value is the mean of four replicated laboratory analyses of each soil sample amended with stover of the same portion of 3 different corn plants from each location.
- Means in the same row followed by different letters are significantly different according to *post hoc* Tamhane's method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between soil samples amended with stover of the same plant portions of isogenic Bt vs. non-Bt corn hybrids.

Table 6. Means of soil total carbon (g C kg^{-1} soil) in samples amended with stover of the upper, middle and lower portions of DKC34-11(Bt) and DKC34-1(non-Bt) corn hybrids grown at Snook, TX after 223 days of incubation.

Plant Portion	Soil Total Carbon (g C kg^{-1} soil) HFC DKC34-11(Bt) Mean ± SD	 HFC DKC34-10(non-Bt) Mean ± SD
Upper	15.3 ± 0.2 (a)	15.3 ± 0.2 (a)
Middle	15.3 ± 0.1 (a)	15.3 ± 0.1 (a)
Lower	15.2 ± 0.1 (a)	15.3 ± 0.2 (a)

- (*) Difference between total soil C concentrations of samples amended with stover and the averaged total soil C concentration of 8 unamended soil samples.
- Each value is the mean of four replicated laboratory analyses of each soil sample amended with stover of the same portion of 3 different corn plants from each location.
- Means in the same row followed by different letters are significantly different according to *post hoc* Tamhane's method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between soil samples amended with stover of the same plant portions of isogenic Bt vs. non-Bt HFC corn hybrids.

The possibility of an increased accumulation of soil C, even a very small one, would represent a substantial benefit in terms of C sequestration since in 2007 approximately 47 million acres were planted to Bt-modified corn in the USA (USDA, 2008a). Nevertheless, because the differences were so small, in the order of 0.07%, they could be easily offset by the effects of soil characteristics and management, environmental conditions and normal variations of the different corn hybrid lines grown in the field. Furthermore, the differences in the decomposition rate of Bt vs. non-Bt corn plants observed in this research might also be attributed to the particular microbial communities present in the soil utilized for these experiments. The process of C mineralization by the decomposer populations is not only limited by the chemical and physical properties of the degradable substrates, but also by the soil properties and, particularly by the selective substrate utilization as a result of the adaptation to the habitat conditions of the decomposer microorganisms (Ekschmitt et al., 2008).

The results of this research indicated that modifications in soil C accumulation due to residue degradation of Bt-modified corn plants are not likely to occur. However, since there was a trend towards slower C mineralization of Bt-corn stover which was in accordance with the results of several other researchers (Masoero et al., 1999; Saxena and Stotzky, 2001; Hopkins and Gregorich, 2003; Flores et al., 2005; Fang et al., 2007), further studies, including measurements under field conditions, are required in order to assess the potential difference in C sequestration between Bt and non-Bt corn plants as modified by corn variety and environmental conditions.

SOIL NITROGEN CONCENTRATION

Overall, there was a slight trend for more mineral N being present in soil samples amended with stover of the Bt hybrids than in samples amended with non-Bt-containing stover (Table 7) of both HFC and non-HFC hybrids. However, the observed trend was not consistent across location and plant portions (Tables 8 and 9), and the differences in soil mineral N concentration were not statistically significant except for the difference between the lower portions of Bt vs. non-Bt plants of the non-HFC hybrid line grown at Snook, TX (Table 8).

Table 7. Overall means of nitrogen mineralized (mg N kg^{-1} soil) in soil samples amended with stover of non-HFC and HFC Bt and non-Bt corn hybrids grown at College Station and Snook, TX after 223 days of incubation.

	Soil Mineralized Nitrogen (mg N kg^{-1} soil))	
Location	**DKC69-71(Bt) Mean ± SD**	**DKC69-72(non-Bt) Mean ± SD**
College Station, TX	74.29 ± 12.28 (b)	69.42 ± 10.22 (a)
Snook, TX	75.17 ± 12.30 (a)	74.99 ± 10.35 (a)
Snook, TX	**HFC DKC34-11(Bt) Mean ± SD**	**HFC DKC34-10(non-Bt) Mean ± SD**
	77.33 ± 9.41 (a)	74.79 ± 11.83 (a)

- Each value is the mean of four replicated laboratory analyses of each soil sample amended with stover of the same portion of 3 different corn plants from each location when data of the three different plant portions was pooled.
- Means in the same row followed by different letters are significantly different according to *post hoc* Tukey's method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between soil samples amended with stover of isogenic Bt vs. non-Bt corn hybrids.

Table 8. Means of nitrogen mineralized (mg N kg^{-1} soil) in soil samples amended with stover of the upper, middle and lower portions of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX after 223 days of incubation.

		Soil Mineralized Nitrogen (mg N kg^{-1} soil)	
Location	**Plant Portion**	**DKC69-71(Bt) Mean ± SD**	**DKC69-72(non-Bt) Mean ± SD**
College Station TX	Upper	80.34 ± 3.59 (a)	70.59 ± 7.17 (a)
	Middle	81.10 ± 5.61 (a)	73.40 ± 8.87 (a)
	Lower	81.11 ± 4.79 (a)	83.35 ± 5.59 (a)
Snook TX	Upper	81.21 ± 5.17 (a)	82.31 ± 6.86 (a)
	Middle	74.91 ± 8.75 (a)	74.89 ± 7.20 (a)
	Lower	75.29 ± 7.42 (a)	76.31 ± 8.09 (b)

- Each value is the mean of four replicated laboratory analyses of each soil sample amended with stover of the same portion of 3 different corn plants from each location.
- Means in the same row followed by different letters are significantly different according to *post hoc* Tamhane's method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between soil samples amended with stover of the same plant portions of isogenic Bt vs. non-Bt corn hybrids.

Table 9. Means of nitrogen mineralized (mg N kg^{-1} soil) in soil samples amended with stover of the upper, middle and lower portions of DKC34-11(Bt) and DKC34-10(non-Bt) corn hybrids grown at Snook, TX after 223 days of incubation.

	Soil Mineralized Nitrogen (mg kg^{-1} soil)	
Plant Portion	**DKC34-11(Bt) Mean ± SD**	**DKC34-10(non-Bt) Mean ± SD**
Upper	70.98 ± 8.81 (a)	65.79 ± 8.39 (a)
Middle	83.61 ± 9.57 (a)	82.09 ± 9.28 (a)
Lower	77.48 ± 5.43 (a)	70.49 ± 7.94 (a)

- Each value is the mean of four replicated laboratory analyses of each soil sample amended with stover of the same portion of 3 different corn plants from each location.
- Means in the same row followed by different letters are significantly different according to *post hoc* Tamhane's method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between soil samples amended with stover of the same plant portions of isogenic Bt vs. non-Bt HFC corn hybrids.

When comparing soil samples amended with the different portions of Bt vs. non-Bt plants, it would appear that there could be a possibility of more mineralized N being available for plants (after a 223-day period of time) in soils amended with the lower portions of Bt-containing stover as affected by variety and variety*location interaction effects. In the case of corn plants grown under non-irrigated conditions at College Station, TX, there was about 4% more available N present in soil samples receiving stover of the lower portion of the non-HFC Bt-plants than in samples amended with the equivalent portion of the isogenic non-Bt corn plants (Table 8). The difference between equivalent portions of the same corn hybrids rose to about 30% for plants grown at Snook, TX (Table 8), and for the lower portion of HFC plants grown at Snook, TX, the difference in available N concentration between soil samples incubated with Bt-containing stover and non-Bt stover was approximately 6%.

Corn plants need relatively high amounts of N to develop properly and even greater N-inputs to reach the high yields expected by the US farmers who apply large quantities of N-fertilizer on a yearly basis to this extensively cultured crop. With the climbing price of N-fertilizers, i.e. anhydrous ammonia price rose 44% between 2007 and 2008 (USDA, 2008b), the knowledge of the fate of N contained in corn stover that may be available for the next crop becomes of capital importance to improve the accuracy of the amount of N to be applied and the synchronization of the N-supply with the corn plant demands. Given the variability of plant N concentration and soil N release resulting from the interactions of the characteristics of each particular hybrid line and field conditions, it would be desirable to determine on-site the available soil N concentration derived from Bt or non-Bt corn residues, in order to adjust the quantity and timing of the N-fertilizer to be applied (in accordance with prevailing climatic conditions), to compensate for lower N concentrations or to take advantage of higher N concentrations while maximizing the farmers' return and minimizing the risks of N losses through denitrification, leaching, and run-off.

Although the differences observed in soil N concentration due to variety, location, Bt-trait and plant portion were not consistent and thus no particular conclusions could be drawn, the trend observed in the lower portions of the corn plants deserves further investigation including the analysis of its interactions with soil type (field site) and management, natural variations between different maize lines, and environmental conditions as they could be useful when evaluating corn stover tillage or removal for ethanol production.

STOVER COMPOSITION

Stover Carbon Concentration

There were no statistically significant differences in total stover C concentration due to hybrid line (HFC vs. non-HFC), location, or Bt-trait except for a small (~2%) difference between stovers of the upper portions of the Bt vs. non-Bt non-HFC plants grown under irrigation at Snook, TX (Tables 10 and 11). Since no significant differences in stover C concentration matched the trend observed in cumulative CO_2 evolved, I expected to perhaps find variations in the proportional distribution of the plant tissue components, such as a higher proportion of soluble carbohydrates in the stover of the DKC69-72(non-Bt) plants.

Table 10. Means of total carbon concentration (g kg^{-1}) in stover of the upper, middle and lower portions of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX.

		Total Carbon Concentration (g C kg^{-1} stover)	
Location	Plant Portion	DKC69-71(Bt) Mean ± SD	DKC69-72(non-Bt) Mean ± SD
College Station TX	Upper	394.80 ± 0.15 (a)	391.60 ± 0.06 (a)
	Middle	413.00 ± 0.50 (a)	409.00 ± 0.35 (a)
	Lower	395.10 ± 1.44 (a)	383.30 ± 0.75 (a)
Snook TX	Upper	402.10 ± 0.08 (b)	392.20 ± 0.10 (a)
	Middle	405.60 ± 0.71 (a)	406.20 ± 0.08 (a)
	Lower	388.60 ± 0.16 (a)	382.50 ± 0.24 (a)

- Each value is the mean of three replicated laboratory analyses of each stover sample. Stover from the same portion of 3 different plants of the same hybrid grown at the same location had been pooled.
- Means in the same row followed by different letters are significantly different according to *post-hoc* Tamhane's method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between the same plant portions of isogenic Bt vs. non-Bt corn hybrids grown at the same location.

Table 11. Means of total carbon concentration (g kg^{-1}) in stover of the upper, middle and lower portions of high fermentable corn hybrids DKC34-11(Bt) and DKC34-10(non-Bt) grown at Snook, TX.

	Total Carbon Concentration (g C kg^{-1} stover)	
Plant Portion	DKC34-11(Bt) Mean ± SD	DKC34-10(non-Bt) Mean ± SD
Upper	389.30 ± 0.14 (a)	393.30 ± 0.45 (a)
Middle	398.50 ± 0.38 (a)	407.70 ± 0.15 (a)
Lower	385.00 ± 0.18 (a)	400.50 ± 0.21 (a)

- Comparisons are made between the same plant portions of isogenic Bt vs. non-Bt HFC corn hybrids.
- Each value is the mean of four replicated laboratory analyses of the same portion of 3 different corn plants.
- Means in the same row followed by different letters are significantly different according to *post-hoc* Tamhane's method (equal variances not assumed) ($p<0.05$).

Stover Nitrogen Concentration

There was a statistically non significant but consistent trend of about 9% difference in the overall N concentration between stover of the Bt-modified and non-Bt corn plants (Table 12). At the College Station field site, the N concentration in stover of the non-HFC Bt-plants was higher (~9%) than that of the isogenic non-Bt plants. The inverse relation was observed at the irrigated field in Snook where the stover of the non-HFC Bt-plants contained less (~9%) N than stover of the isogenic non-Bt plants. Stover of the HFC Bt-hybrids grown at Snook had approximately 9% more N than stover of the isogenic non-Bt plants grown at the same location (Table 12). This difference is very small if we consider that it is 9% of a ~1% N concentration. Therefore, the practical significance of these results is at best small.

Table 12. Overall means of total nitrogen concentration (g kg^{-1}) in stover of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX and DKC34-11(Bt) and DKC34-10(non-Bt) hybrids grown at Snook, TX.

	Total Nitrogen Concentration (g N kg^{-1} stover)	
Location	**DKC69-71(Bt) Mean ± SD**	**DKC69-72(non-Bt) Mean ± SD**
College Station, TX	9.8 ± 0.5 (a)	8.8 ± 0.6 (a)
Snook, TX	9.8 ± 0.6 (a)	10.7 ± 0.7 (a)
Snook, TX	**HFC DKC34-11(Bt) Mean ± SD**	**HFC DKC34-10(non-Bt) Mean ± SD**
	9.8 ± 0.7 (a)	9.0 ± 0.5 (a)

- Each value is the mean of the pooled data for the three different plant portions. Data correspond to three replicated laboratory analyses of each stover sample. Stover from the same portion of 3 different plants of the same hybrid grown at the same location had been combined.
- Means in the same row followed by different letters are significantly different according to *post-hoc* Tukey's method (equal variances assumed) ($p<0.05$).
- Comparisons are made between isogenic Bt vs. non-Bt corn plants.

Stover of the non-HFC non-Bt plants grown at Snook contained approximately 18% more total N than the same plants grown at College Station, which contrasts with the stover of the isogenic Bt-modified plants that had the same N concentration irrespective of the site where they were grown (Table 12). It is known that N availability and source affect its accumulation in corn grain and stover (Chevalier and Schrader, 1977), and that maize varieties may vary in the composition of stover due to their diverse ability to extract nutrients, resistance to stress, and ability to translocate nutrients to the grain (Chevalier and Schrader, 1977; Ma and Subedi, 2005; Bal, 2006; Subedi and Ma, 2007). Thus, when corn hybrids are grown under similar conditions, variations in the composition of stover are accounted for primarily by varietal characteristics. Since the hybrid line (non-HFC) was the same, the differences in the total N concentration between the non-Bt/Bt plants of this hybrid line might be related to the Bt-trait, or rather to a Bt-trait*location interaction effect. These results agree with the findings of Ma and Subedi (2005) who studied N concentration and partitioning in 7 different Bt/non-Bt pairs and reported that, without pest pressure and at the same maturity stage, the non-Bt hybrids accumulated more N than the Bt-modified plants of similar varieties.

It was rather surprising to find that the Bt plants had similar total N concentrations regardless of growing environment and variety, particularly when the HFC hybrid line had been engineered to have increased concentration of soluble substances and enhanced degradability of the above-ground parts of the plant. However, to assess if this could be connected to the Bt-insertion or was the result of the combined effects of corn variety, environmental conditions and laboratory procedures, would require further studies including large numbers of non-Bt/Bt-modified corn varieties grown under various climatic, and N- and water-availability conditions.

I also noticed that under irrigation the Bt-plants concentrated N in the opposite portions compared to non-Bt plants. The partitioning of N along the various portions of the non-Bt plants was the same regardless of variety and location, namely: upper > middle > lower. On the contrary, the partitioning of stover N of the Bt-modified plants was the same as that of the non-Bt plants when grown at College Station where plants suffered periods of insufficient water availability; but showed an inverse order (upper < middle < lower) when grown under irrigation at Snook (Table 13). Variety did not appear to affect N-partitioning of the Bt-modified hybrids as the plants of both, the HFC and the non-HFC varieties, grown at Snook showed the same N-partitioning order (upper < middle < lower) that was opposite to that of the non-Bt maize plants of the same hybrid lines (upper > middle > lower) (Tables 13 and 14).

Table 13. Means of total nitrogen concentration (g kg^{-1}) in stover of the upper, middle and lower portions of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX.

		Total Nitrogen Concentration (g N kg^{-1} stover)	
Location	**Plant Portion**	**DKC69-71(Bt) (Mean ± SD)**	**DKC69-72(non-Bt) (Mean ± SD)**
College Station, TX	Upper	11.0 ± 0.1 (a)	10.8 ± 0.6 (a)
	Middle	9.0 ± 0.8 (a)	8.5 ± 0.2 (a)
	Lower	7.0 ± 0.2 (a)	6.4 ± 0.4 (a)
Snook, TX	Upper	8.6 ± 0.1 (a)	11.8 ± 0.5 (b)
	Middle	9.0 ± 0.4 (a)	11.2 ± 0.2 (b)
	Lower	12.1 ± 0.5 (b)	8.6 ± 0.4 (a)

- Each value is the mean of three replicated laboratory analyses of each stover sample. Stover from the same portion of 3 different plants of the same hybrid grown at the same location had been pooled.
- Means in the same row followed by different letters are significantly different according to *post-hoc* Tamhane’s method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between the same plant portions of isogenic Bt vs. non-Bt corn hybrids.

Table 14. Means of total nitrogen concentration (g kg^{-1}) in stover of the upper, middle and lower portions of DKC34-11(Bt) and DKC34-10(non-Bt) corn hybrids grown at Snook, TX.

		Total Nitrogen Concentration (g N kg^{-1} stover)	
Location	**Plant Portion**	**HFC DKC34-11(Bt) (Mean ± SD)**	**HFC DKC34-10(non-Bt) (Mean ± SD)**
Snook, TX	Upper	7.1 ± 0.3 (a)	11.1 ± 0.3 (b)
	Middle	11.9 ± 0.1 (b)	10.0 ± 0.7 (a)
	Lower	12.5 ± 1.1 (b)	6.9 ± 0.7 (a)

- Each value is the mean of three replicated laboratory analyses of each stover sample. Stover from the same portion of 3 different plants of the same hybrid grown at the same location had been pooled.
- Means in the same row followed by different letters are significantly different according to *post-hoc* Tamhane's method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between the same plant portions of isogenic Bt vs. non-Bt corn hybrids.

It has been reported (Ma and Subedi, 2005) that when European corn borer (*Ostrinia nubilalis*) infestation is low to moderate, non-Bt plants tended to accumulate more N in the grain than the Bt-counterparts that tended to accumulate more N in the stover. This might explain the N-partitioning of the non-Bt hybrids since maize plants commonly mobilize N from the stalks to the grain (Ta and Weiland, 1992), and also the N-partitioning of the Bt-corn varieties that were engineered to resist the European corn borer attack by developing sturdier stalks and killing the lepidoptera larvae on ingestion of the Bt-protein. As a consequence, the Bt-trait might indirectly affect N-partitioning due to an accumulation of the Bt-protein in the middle and lower portions of the plants. However, only the Bt-plants grown at Snook, where there was no sign of infestation (neither corn borer nor any other pest or disease), showed this partitioning pattern, while corn plants of similar Bt-hybrids grown near College Station accumulated more N in the upper portion of the plants. In order to understand why this might have occurred, future studies should include the analysis of the Cry-protein concentration in the different portions of the plant including grain, kernels and stover.

Starch

The chemical analysis of the plant tissues showed a similar overall concentration of starch in the stover of both the non-Bt and Bt-modified plants of the non-HFC hybrid line, irrespective of the location where they were grown (Table 15). There appeared to be a hybrid line*Bt interaction because the non-Bt plants of the HFC corn variety had a significantly higher overall concentration of starch (~16 %) than the Bt-modified plants of this variety (Table 15). Furthermore, the HFC Bt hybrids had an overall starch content about 15% higher than the non-HFC Bt-corn plants while the HFC non-Bt hybrids contained approximately 30% more starch than the non-HFC non-Bt plants (Table 15).

Table 15. Overall means of starch concentration (g kg^{-1}) in stover of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX and high fermentable corn hybrids DKC34-11(Bt) and DKC34-10(non-Bt) grown at Snook, TX.

Location	**Starch Concentration (g starch kg^{-1} stover)**	
	DKC69-71(Bt) Mean ± SD	**DKC69-72(non-Bt) Mean ± SD**
College Station, TX	380.5 ± 30.4 (a)	363.9 ± 66.4 (a)
Snook, TX	385.4 ± 46.3 (a)	367.7 ± 71.0 (a)
Snook, TX	**HFC DKC34-11(Bt) Mean ± SD**	**HFC DKC34-10(non-Bt) Mean ± SD**
	445.1 ± 38.2 (b)	518.5 ± 57.3 (c)

- Each value is the mean of the pooled data for the three different plant portions. Data correspond to three replicated laboratory analyses of each stover sample. Stover from the same portion of 3 different plants of the same hybrid grown at the same location had been combined.
- Means in the same row or column followed by different letters are significantly different according to *post-hoc* Tamhane’s method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between isogenic Bt vs. non-Bt corn plants.

Starch partitioning appeared to be affected by Bt-insertion, HFC-trait, water-availability and their interactions because a) the starch partitioning was different between non-HFC non-Bt and Bt-modified corn plants when grown under irrigated conditions but was similar when grown under a water-deficient regime; b) water availability did not affect the starch partitioning of non-HFC non-Bt plants but modified that of non-HFC Bt-modified corn plants; c) the partitioning of both irrigated and water-deficient non-HFC Bt-plants differed from that of the irrigated HFC Bt-hybrids, and d) the starch partitioning differed between non-Bt and Bt-modified plants of the irrigated HFC hybrids (Tables 16 and 17).

Table 16. Means of starch concentration (g kg^{-1}) in stover of the upper, middle and lower portions of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX and DKC34-11(Bt) and DKC34-10(non-Bt) hybrids grown at Snook, TX.

		Starch Concentration (g starch kg^{-1} stover)	
Location	**Plant Portion**	**Mean ± SD DKC69-71(Bt)**	**Mean ± SD DKC69-72(non-Bt)**
College Station TX	Upper	376.6 ± 4.0 (a)	434.8 ± 4.2 (b)
	Middle	366.1 ± 4.3 (c)	417.5 ± 6.1 (d)
	Lower	347.3 ± 2.5 (e)	280.8 ± 2.4 (f)
Snook, TX	Upper	329.6 ± 2.8 (a)	407.2 ± 3.3 (b)
	Middle	437.9 ± 2.0 (c)	433.1 ± 3.3 (c)
	Lower	388.8 ± 3.7 (d)	263.0 ± 2.0 (e)
		HFC DKC34-11(Bt)	**HFC DKC34-10(non-Bt)**
	Upper	442.7 ± 2.4 (a)	498.5 ± 2.9 (b)
	Middle	523.9 ± 2.6 (c)	542.3 ± 2.8 (d)
	Lower	413.0 ± 2.2 (e)	570.4 ± 2.0 (d)

- Each value is the mean of three replicated laboratory analyses of each stover sample. Stover from the same portion of 3 different plants of the same hybrid grown at the same location had been pooled.
- Means in the same row or column followed by different letters are significantly different according to *post-hoc* Tukey’s method (equal variances assumed) ($p<0.05$).

Table 17. Starch partitioning in stover of the upper, middle and lower portions of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX, and DKC34-11(Bt) and DKC34-10(non-Bt) hybrids grown at Snook, TX.

		Starch Partitioning	
Water Regime	**Variety**	**Bt-modified corn**	**non-Bt corn plants**
non-irrigated	**non-HFC**	L < M < U	L < M < U
irrigated	**non-HFC**	U < L < M	L < M < U
irrigated	**HFC**	L < U < M	U < M < L

HFC = high fermentable corn U = upper plant portion **M** = middle plant portion **L** = lower plant portion

Thus, the differences in C mineralization could not be explained in terms of starch quantity, however, differences in the quality or degradability of the starch or other carbohydrates might explain the increased CO_2 flux of the DKC69-72 (non-Bt) plants grown at Snook, TX, and might also explain why the higher (~15%) overall starch concentration of the HFC non-Bt hybrid (Table 15) did not appear to affect its degradation in soil (see Table 2). The degradability of starch might have been affected by an increased amylopectin:amylose ratio since amylopectin is water soluble and easily degradable (Wu et al., 2006) while amylose is more difficult to degrade (Jaeger et al., 2006), and this ratio varies between hybrid lines, tissue, and developmental stages and is modified by environmental conditions during plant growth (Dang and Boyer, 1988). Furthermore, temperature and water availability during corn plant development induce changes in the chain length and branching pattern of the amylopectin molecules (Dang and Boyer, 1988) so an increase in degradability could have been caused by either an increased concentration of amylopectin or changes in its molecular structure, or both. Variations in starch quantity and quality in maize stover are common because of its transient nature as it is synthesized in the leaves during the day and is degraded at night to provide C for non-photosynthetic metabolism (Jung and Sheaffer, 2004). In addition, the decomposition of starch in soils can be altered by many other factors, or rather the combination of many interconnected variables including modified amylopectin-amylose ratio (Wu et al., 2006), and different spatial distribution of the starch synthases and starch branching enzymes that can enhance or decrease the accessibility to amylolytic enzymes (Thompson, 2000). The number of variables is further increased in HFC hybrids because their higher starch concentrations are achieved by modifying genes coding for modified expression of various starch synthases, starch branching enzymes and/or starch debranching enzymes while their higher degradability is obtained by altering the crystallinity of starches to increase the accessibility to enzymatic digestion (Agbios, 2008; Muller-Langer et al., 2008), and/or by the production of a bacterial-derived heat-tolerant/thermo-stable alpha-amylase which catalyses the breakdown of starch into smaller molecules, mostly sugars, to replace the external addition of microbes or microbially produced enzyme during its fermentation for ethanol production. Although the last trait was engineered to produce the enzyme only in the kernel (with storage in the endoplasmic reticulum of grain), the alpha-amylase has also been detected in root, leaf and other plant tissues (Agbios, 2008; Muller-Langer et al., 2008).

The biosynthesis and degradation of starch in storage tissue (grain) and transient starches accumulated in leaves and non-storage tissues, amylose biosynthesis, and amylopectin biosynthesis are all encoded by different genes. In addition, the large number of enzymatic reactions in the pathway of starch synthesis and sugar metabolism involve isoenzymes (debranching enzymes) encoded by other different genes, resulting in modifications of the same enzyme activity within a given tissue and between tissues and/or modifications of the activity of different enzymes in a tissue specific manner. Thus, elucidating if the Bt-gene actually induced alterations in starch production or structure, and the nature of the interactions among environmental factor(s), Bt-insertion, hybrid line trait(s), soil physico-chemical characteristics and microbial biomass is a very complicated matter that will require many more experiments and ample inter-disciplinary collaboration.

Subsequent to the completion of this research, it was learned that due to the characteristics of the new corn hybrids, the analytical method used for starch determinations may have overestimated the starch content due to the possible detection of other oligosaccharides. This might partially explain the elevated starch values reported here.

Cellulose

Although statistically significant, differences in the cellulose concentrations of the stovers were inconsistent and therefore, no particular conclusions were drawn in relation to cellulose concentration or distribution in corn plant tissue as modified by location, hybrid or Bt-trait effect (Table 18).

Table 18. Composition of stover of the upper, middle and lower portions of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX and DKC34-11(Bt) and DKC34-10(non-Bt) hybrids grown at Snook, TX.

			Concentration (g kg^{-1} stover) Means ± SD			
Location	**Hybrid**	**Plant Portion**	**Starch**	**Hemicellulose**	**Cellulose**	**Lignin**
College Station, TX	DKC69-71 (Bt)	Upper	376.6 ± 4.0	238.3 ± 13.6	366.9 ± 12.8	40.0 ± 1.4
		Middle	366.1 ± 4.3	261.9 ± 54.9	410.1 ± 51.2	27.5 ± 1.4
		Lower	347.3 ± 2.5	211.0 ± 41.3	403.5 ± 47.1	48.3 ± 2.3
	DKC69-72 (non-Bt)	Upper	434.8 ± 4.2	253.8 ± 27.5	337.2 ± 21.6	53.9 ± 0.5
		Middle	417.5 ± 6.1	301.0 ± 3.5	394.3 ± 3.9	27.9 ± 2.1
		Lower	280.8 ± 2.4	212.0 ± 2.5	442.2 ± 7.1	41.2 ± 0.9

Table 18. (continued)

Location	Hybrid	Plant Portion	Concentration (g kg^{-1} stover) Means ± SD			
			Starch	**Hemicellulose**	**Cellulose**	**Lignin**
Snook, TX	DKC69-71 (Bt)	Upper	329.6 ± 2.8	215.1 ± 27.2	400.1 ± 32.4	34.6 ± 2.3
		Middle	437.9 ± 2.0	238.7 ± 14.4	393.5 ± 20.1	22.8 ± 0.9
		Lower	388.8 ± 3.7	225.4 ± 16.4	341.5 ± 13.8	64.2 ± 1.3
	DKC69-72 (non-Bt)	Upper	407.2 ± 3.3	220.8 ± 8.8	358.9 ± 14.6	45.6 ± 2.3
		Middle	433.1 ± 3.3	304.0 ± 23.8	341.1 ± 25.1	30.6 ± 0.5
		Lower	263.0 ± 2.0	204.8 ± 5.8	394.6 ± 9.2	52.4 ± 1.0
	DKC34-11 (Bt)	Upper	442.7 ± 2.4	203.0 ± 38.2	407.2 ± 34.0	69.0 ± 0.7
		Middle	523.9 ± 2.6	238.1 ± 8.8	380.0 ± 11.3	54.8 ± 1.2
		Lower	413.0 ± 2.2	199.3 ± 40.1	403.8 ± 40.6	53.2 ± 0.8
	DKC34-10 (non-Bt)	Upper	498.5 ± 2.9	199.4 ± 31.9	434.9 ± 28.5	29.9 ± 0.4
		Middle	542.3 ± 2.8	236.4 ± 6.0	394.4 ± 5.1	33.9 ± 1.2
		Lower	570.4 ± 2.0	323.1 ± 0.5	243.9 ± 43.8	28.1 ± 0.2

Variations in the concentration and distribution of cellulose can result from the combination of genetic and environmental factors that alter not only the cellulose synthesis but also post-synthetic cellulose deposition. Such variations are still unpredictable since the enzymes and genes involved in cellulose synthesis in maize plants have not been completely characterized. Presently, 12 members of the cellulose synthase (CesA) gene family of maize have been isolated and it has been found that their expression extends to multiple tissues (Anterola and Lewis, 2002). Appenzeller et al. (2004), reported that 3 of the 12 genes appeared to be coordinately expressed but the remaining genes showed overlapping expression to varying degrees, making it impossible, at the present state of the art, to distinguish pleiotropy from tight linkage when comparing traits.

Hemicellulose

The overall concentration of hemicellulose in stover of the different Bt-modified hybrids grown at both locations was lower than hemicellulose concentration in stover of the non-Bt isogenic counterpart. It has been found that hybrids with lower neutral detergent fiber (NDF) and lower lignin had higher starch concentration (Barriere et al., 2004), and that stover of Cry1Ab modified corn hybrids that had lower hemicellulose also had higher sugars and starch concentrations (Rossi et al., 2003). However, no clear correlation among Bt-trait, hemicellulose, lignin and/or starch concentrations was found in the present study (Table 18).

Lignin

Because the percentage of total C concentration in stover of the HFC non-Bt and Bt-modified plants was very similar, it was expected that the higher starch fraction would have been balanced by different proportions of other structural substances. The chemical analysis of the stover confirmed that the non-HFC hybrids that had no significant differences in the overall starch concentration (Table 16) had similar amounts of cellulose, hemicellulose and lignin (Table 17). Accordingly, the analysis of the HFC stover showed that the lower amount of starch of the DKC34-11(Bt) compared with the non-Bt isogenic hybrid, was compensated by an approximately two-fold increase in the amount of lignin (Table 19).

Thus, the results of the experiments carried out with the non-Bt/Bt-modified plants of the non-HFC corn hybrid line are in agreement with several researchers (Folmer et al., 2002; Jung and Sheaffer, 2004; Mungai et al., 2005), who found no significant differences in the lignin concentration or in the degradation of the residues of non-Bt and Bt-modified isogenic corn hybrids in soil. In contrast, the results of the experiments with the HFC line (Figure 8) are in accord with the findings of many other researchers (Masoero et al., 1999; Saxena and Stotzky, 2001; Hopkins and Gregorich, 2003; Flores et al., 2005; Fang et al., 2007) who reported a higher concentration of lignin in Bt-modified corn plants than in the non-Bt plants of the corresponding isogenic corn hybrids that they considered responsible for the observed decrease in CO_2 evolution. This does not necessarily imply that the factor(s) required for the production of the higher/lower lignin concentration in the Bt/non-Bt plants of the non-HFC line (Table 19) did not exist. They may have existed but were dormant, recessive, or had been "silenced" by another trait of the particular corn hybrid line under study.

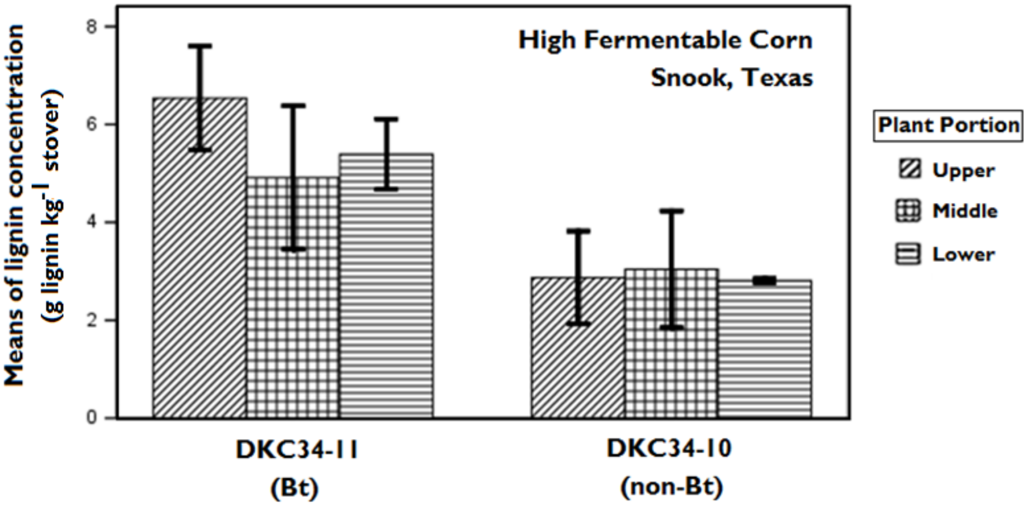

Fig. 8. Means of lignin concentration (g kg^{-1}) in stover of DKC34-11(Bt) and DKC34-10(non-Bt) high fermentable corn hybrids grown at Snook, TX. Error bars represent ± 2 SD.

Table 19. Means of lignin concentration (g kg^{-1}) in stover of the upper, middle and lower portions of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX.

		Lignin Concentration (g lignin kg^{-1} stover)	
Location	Plant Portion	DKC69-71(Bt) Mean ± SD	DKC69-72(non-Bt) Mean ± SD
College Station TX	Upper	40.0 ± 1.4 (a)	53.9 ± 0.5 (b)
	Middle	27.5 ± 1.4 (a)	27.9 ± 2.1 (a)
	Lower	48.3 ± 2.3 (a)	41.2 ± 0.9 (a)
Snook TX	Upper	34.6 ± 2.3 (a)	45.6 ± 2.3 (b)
	Middle	22.8 ± 0.9 (a)	30.6 ± 0.5 (b)
	Lower	64.2 ± 1.3 (a)	52.4 ± 1.0 (b)

- Each value is the mean of four replicated laboratory analysis of the same portion of 3 different corn plants from each location.
- Means in the same row followed by different letters are significantly different according to *post hoc* Tamhane's method (equal variances not assumed) ($p<0.05$).
- Comparisons are made between the same plant portions of isogenic Bt vs. non-Bt corn hybrids.

Furthermore, the present study showed that, in spite of the higher lignin concentration of the HFC Bt-modified plants (Table 20, Figure 8), there was a trend towards a lower CO_2 evolution of the Bt-modified stovers but there were no statistically significant differences in cumulative CO_2 evolution at the end of the 223-day incubation period (Table 4) or any other incubation period (data not shown).

Table 20. Means of lignin concentration (g kg^{-1}) in stover of the upper, middle and lower portions of high fermentable corn hybrids DKC34-11(Bt) and DKC34-10(non-Bt) grown at Snook, TX.

	Lignin Concentration (g lignin kg^{-1} stover)	
Plant Portion	HFC DKC34-11(Bt) Mean ± SD	HFC DKC34-10(non-Bt) Mean ± SD
Upper	69.0 ± 0.7 (b)	29.9 ± 0.4 (a)
Middle	54.8 ± 1.2 (b)	33.9 ± 1.2 (a)
Lower	53.2 ± 0.8 (b)	28.1 ± 0.2 (a)

- Comparisons are made between the same plant portions of isogenic Bt vs. non-Bt HFC corn hybrids.
- Each value is the mean of four replicated laboratory analyses of the same portion of 3 different corn plants.
- Means in the same row followed by different letters are significantly different according to *post-hoc* Tamhane's method (equal variances not assumed) ($p<0.05$).

The effect of the higher lignin concentration on the cumulative CO_2 evolution might have been masked by the engineered enhanced degradability of the structural components of the HFC hybrids. There was also a possibility that the modifications introduced in the HFC hybrids also included an increased degradability of their lignin, and that this trait was enhanced by its interaction with the Bt-insertion as previously observed by Escher et al. (2000) who reported a faster rate of lignin decomposition of the transgenic vs. the non-transgenic corn residues.

CONCLUSIONS

The objectives for this study were to determine if Bt-corn hybrid stover would decompose in soil at a slower rate than the residues of the corresponding non-Bt isogenic plants, verify if the Bt-insertion was associated with changes in the structural chemistry of maize biomass, and compare C and N mineralization of (1) three different portions of isogenic Bt-modified and non-Bt plants of a corn variety grown at two different locations, and (2) three different portions of isogenic Bt-modified and non-Bt plants of two different hybrid lines, one of them, a high fermentable corn variety, grown in the same field.

The results of the experiments indicated a possible Bt-trait*variety interaction effect as less C was decomposed at day one of laboratory incubations of soil samples containing non-HFC non-Bt-corn residues, and a Bt-trait*variety*location (water-availability) because no statistically significant differences were observed between soil samples amended with residues of non-HFC non-Bt and Bt-corn plants grown at College Station, or HFC non-Bt vs. Bt-modified plants grown at Snook, yet there was a consistently significant difference in cumulative CO_2 evolved between soil samples receiving stover of non-HFC non-Bt and Bt-modified hybrids, at all the incubation periods tested. Although not all differences in cumulative amounts of C mineralized at different incubation periods from stover of different portions of HFC and non-HFC Bt vs. non-Bt plants were statistically significant, there was an overall trend for the soil samples amended with stover of the Bt-modified hybrids of the two varieties to mineralize less (15% for HFCs and 10% for non-HFCs) C, as measured by CO_2 evolution, than those receiving residues of the corresponding non-Bt isogenic plants.

There were no statistically significant differences in soil total C consistent with the differences observed in CO_2 evolution. The differences in soil N were not consistent with C mineralization of plant residues (measured as cumulative CO_2 evolved) and were significant only for the soil samples amended with stover of Bt/non-Bt plants of the non-HFC variety grown under irrigated conditions, however, there was a trend towards higher soil mineral N accumulation in the samples amended with Bt-corn residues. The structural composition of the corn plants was affected by the Bt-trait, HFC-trait, location and their interactions, however, differences in total C, total N, and biomass fractions of the initial Bt and non-Bt hybrid stover did not correspond to differences in decomposition.

It appears that there might be a potential to sequester more C under Bt-corn cultivation, however, it could be easily offset by soil characteristics, environmental conditions and normal variations of the diverse corn lines. Based on the results of this study, it may be concluded that the cultivation of Bt-modified maize lines is not likely to affect soil C or N dynamics compared with the cropping of non-Bt hybrids. On the other hand, HFC hybrids may have a greater impact on soil C or N dynamics than non-HFC corn varieties, independent of their Bt-concentration that might add a barely noticeable addition to the HFC-effect.

CHAPTER V

SUMMARY AND CONCLUSIONS

SUMMARY

The purpose of this research was to gain knowledge on the possible influence of the Bt-protein insertion on decomposition of corn residue in soil. The study focused primarily on the comparison of C and N mineralization of corn stover in soil as affected by Bt-trait, environmental (location) conditions, plant portion and HFC-trait; and secondarily on the existence of Bt-related variations in the chemical structure of corn residues that might affect the degradation rate of corn stover in soil and consequently the soil C and N dynamics.

The study showed that the differences observed in the degradation rate (as measured by CO_2 evolution) of Bt vs. non-Bt corn stover were dependent on environmental conditions (irrigated vs. non-irrigated settings) and hybrid variety (HFC vs. non-HFC hybrid lines), and that there were not significant differences in stover degradation in soil between the different portions (upper, middle, and lower) of the HFC corn plants.

During the first day of incubation, about 3X more CO_2 evolved from soil samples containing stover of the non-Bt-modified plants of the non-HFC variety (DKC69-72) than from soil samples amended with the isogenic Bt-modified corn plants (DKC69-71) grown under irrigation at Snook. This was not observed in the soil samples amended with stover of the upper and middle portions of corn plants of the same variety grown under non-irrigated conditions at College Station, and only the soil samples receiving stover of the lower portion of DKC69-72(non-Bt) corn plants evolved more CO_2 (about 2.5X more) than samples receiving the equivalent portion of the DKC69-71 (Bt) plants. At the end of the 223-day incubation period, the differences were still significant but had decreased to only about 10 to 15% higher cumulative $^{223}CO_2$ in soil samples amended with Bt-free stover samples of the irrigated non-HFC hybrids and the lower portion of the non-irrigated plants of the same variety. This difference in CO_2 evolution was not statistically significant for soils samples amended with stover of non-Bt/ Bt-modified corn plants of the high fermentable corn variety (grown under irrigation at Snook) although there was a trend for about 15% less CO_2 being evolved from Bt-containing stover.

At the end of the 223-day incubation period, there were no statistically significant differences in total soil C content between samples amended with stover of the Bt-modified or non-Bt corn plants of any of the two corn varieties (HFC and non-HFC) used in this study. There was also no noticeable trend in soil C concentration that matched the trend observed in cumulative CO_2 evolution or stover C. Actually, the differences in soil C content were so small (< 0.07%) that, according to the results of this research, no differences in soil C sequestration would be expected to arise due to the Bt-content of corn stover.

After 223 days of incubation, there appeared to be a trend indicating that there was a possibility of more soil N being available for plant uptake in samples amended with Bt-containing stover than in samples receiving Bt-free stover of both HFC and non-

HFC hybrid lines, however, the observed trend was not consistent across location and plant portions, and more importantly, the differences in soil mineral N concentrations were not statistically significant except for only one comparison corresponding to the lower portions of Bt versus non-Bt (irrigated) plants of the non-HFC hybrid line grown at Snook. In the case of corn plants grown under non-irrigated conditions at College Station, there was about 4% more available N present in soil samples receiving stover of the lower portion of the non-HFC Bt-plants than in samples amended with the equivalent portion of the isogenic corn plants. The difference between equivalent portions of the same corn hybrids rose to about 30% for plants grown at Snook, and was about 6% for the HFC plants grown at the same location. Due to the actual importance of N-fertilizers costs, as illustrated by the 44% increase in the price of anhydrous ammonia between 2007 and 2008 (USDA, 2008b), small percentages in N availability would represent important savings particularly when growing corn in the US that requires an average input of 180 kg N per hectare, thus, it seems worthwhile to perform further experiments to gain knowledge on the N released in soils by stover of the lower portions of different Bt/non-Bt pairs as it could financially benefit the farmers, and at the same time decrease environmental concerns due to denitrification, leaching and run-off caused by the application in excess of N-fertilizers.

There were differences in N-partitioning between Bt-containing and Bt-free stover that appeared to be possibly affected by the interaction of Bt-trait with environmental conditions and corn variety, a finding that reinforces the need of studying the N dynamics in soils of the different portions of Bt/non-Bt corn plants to be able to take advantage of this knowledge when evaluating N-fertilizer inputs and removal of stover for ethanol production.

There were no significant differences in starch concentration between Bt and non-Bt hybrids of the non-HFC line grown at either location. However, Bt-modified HFC plants had lower starch content (approximately 15%) than the isogenic non-Bt HFC corn plants, and there appeared to be differences in starch partitioning that may have been related to the interaction of the Bt-insertion with other traits of the corn varieties under study.

Environmental conditions affected the partitioning along the plant but not the total lignin content of Bt and non-Bt plants of the non-HFC hybrid corn line. There were no significant differences in the concentration of lignin between Bt and non-Bt plants of the non-HFC variety; however, HFC Bt corn hybrids had twice as much lignin than the isogenic HFC non-Bt plants.

No significant differences were noted in the content of cellulose between Bt versus non-Bt hybrids and HFC versus non-HFC corn plants. The middle part of all the corn hybrids had a higher hemicellulose concentration than either the upper or lower parts of the plant with the only exception of the HFC non-Bt plants that accumulated more hemicellulose in the lower part of the plant.

CONCLUSIONS

Further research is required to ascertain the nature of the delay observed during the first day of incubation in the degradation of Bt-corn residues of the non-HFC variety. The fact that the delay was not observed in the incubations with stover of the Bt-modified HFC hybrid line that was specially engineered to have a higher degradability and content of the more soluble C compounds suggests that one or more of the following may be happening:

1. There may be a pleiotropic effect of the Bt insertion on the chemical or structural composition of the soluble carbohydrates, e.g. (a) a different ratio in the amylopectin/amylose content of starch, (b) a structural or 3-D modification that makes sugars and starch less accessible to the microbes, or (c) both (a) and (b).
2. There may be a specific Bt-effect on certain members of the decomposer cohort such as some enzyme or a secondary metabolite or other corn trait “activated” by the Bt-insertion that remains “dormant” in the non-Bt modified corn plants, which only affects these microbes.
3. The presence or absence of a certain component of the Bt-modified corn stover may result in the development of different microbial communities.

Therefore, further research should include a detailed analysis to verify if (a) there is a Bt-related alteration in the chemical composition or spatial structure of starch and/or other soluble carbohydrates that might explain the delay in the degradation of the Bt-residues; and should such alteration exist, (b) if it is the same in HFC-Bt than non-HFC Bt-modified corn hybrid lines.

If the observed trend in CO_2 evolution from Bt and non-Bt corn stover were due to an altered amylase:amylopectin ratio or 3-D structure of starch, the reduction or delay in C degradation of the Bt-modified residues may have resulted from a bacterial need to “adjust” the production of their extracellular enzymes (i.e., amylases) before they could hydrolyze starch, or to reach a certain concentration before the hydrolytic reaction could start.

This type of alterations in starch composition has already been found in genetically engineered potatoes as compared with the original non-modified potato plants. Studies showed that the modifications in starch were caused by the reduced amount of an endogenous protein (EFSA, 2006).

HFC and non-HFC corn hybrids could have different modifications in their starch or sugar composition that resulted in similar effects on residue degradation. The non-HFC corn hybrids were being tested for their tolerance to the water-deficient climatic conditions usually prevailing in Texas, and it is known that some of the genes that are manipulated to increase drought resistance also increase the content of certain soluble carbohydrates. Conversely, it has been found that genes manipulated to affect sugar metabolism also increase drought resistance (Paul et al., 2001; Garg et al., 2002) which could be the case of the HFC hybrids.

Thus, the results of the suggested research could be useful to clarify the connection between sugar metabolism and drought-resistance in corn plants, leading to the simultaneous improvement of more than one corn trait at the same time.

The HFC hybrid lines used in our experiments are promoted, among other things, for their ability to produce “greener leaves” and a higher biomass for increased benefits when delivering the stover to ethanol plants. The genetic modifications that alter the assimilation and utilization efficiency of nutrients can result in an increased nutrient content in the plants’ tissues (Xiao et al., 2006). For example, tobacco plants engineered to increase their N efficiency through increased ammonium assimilation resulted in plants with greater biomass and leaf soluble protein content compared to the non-transgenic tobacco plants (Oliveira et al., 2002). There is a possibility that this trait interacted with the Bt-trait inducing the differences observed in N-partitioning. Consequently, further studies involving more diverse field soil conditions and hybrids are needed to verify grain and stover N-content and yield at low N availability because these traits could be used to select maize tolerant to low-N soils. This could decrease the N-fertilizer amount to be applied with its derived economic and environmental benefits. The variations observed in stover N content and partitioning due to possible interactions of the Bt-trait with location and HFC/non-HFC traits coupled with the differences observed in soil N concentration points to the convenience of testing soil N available for the crop before recommending the amount and timing of the N-fertilizer to be applied while a better understanding of N partitioning would help evaluating what portions of the corn plant that could be sent for ethanol production could benefit more the soil fertility, protection from erosion and alcohol yield.

On the other hand, the initial decomposition of plant residues in soil depends on the substrate composition, on the soil chemical and physical properties, and on the decomposer microflora resulting from the microbial adaptation to the habitat conditions of each particular soil (Verpoorte et al., 2002; Ekschmitt et al, 2008). Therefore, future studies should also include the characterization of the decomposer microorganisms of the Weswood soil used for the incubations in this research, to avoid confounding the effects of the Bt-trait or genotype-environmental interactions on C and N mineralization of stover with the limitations of the particular decomposers existing in this particular soil. Knowing what differences in the Bt- versus non-Bt derived stover caused the observed differences in soil C and N mineralization, coupled with the knowledge of how the particular community present in the soil used for this study responded to such differences, may reveal predictable trends in microbial community structures that could be applied to other soil types.

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APPENDIX

Table A-1. Carbon mineralization kinetics after soil additions of stover of the upper, middle and lower portions of DKC69-71(Bt) and DKC69-72(non-Bt) corn hybrids grown at College Station and Snook, TX and DKC34-11(Bt) and DKC34-11(non-Bt) hybrids grown at Snook, TX.

Location	Hybrid DKC	Trait	Plant Portion	C_0 (mg g^{-1} soil)	Day7		Day14		Day 21		Day 35		Day 223	
					k (day^{-1})	R^2	k (day^{-1})	R^2	k (day^{-1})	R^2	k (day^{-1})	R^2	k (day^{-1})	R^2
College Station TX	69-71	Bt	Upper	1.97	0.054	0.89	0.038	0.93	0.033	0.92	0.024	0.87	0.005	0.60
	69-72	non-Bt		1.96	0.06	0.93	0.042	0.91	0.030	0.89	0.021	0.84	0.005	0.68
	69-71	Bt	Middle	2.06	0.041	0.83	0.031	0.89	0.026	0.91	0.019	0.87	0.005	0.71
	69-72	non-Bt		2.04	0.052	0.96	0.036	0.94	0.029	0.92	0.021	0.88	0.005	0.64
	69-71	Bt	Lower	1.98	0.034	0.62	0.029	0.85	0.024	0.90	0.018	0.88	0.005	0.74
	69-72	non-Bt		1.92	0.042	0.81	0.032	0.88	0.028	0.91	0.021	0.90	0.007	0.85
Snook TX	69-71	Bt	Upper	2.01	0.047	0.89	0.034	0.92	0.028	0.92	0.021	0.89	0.005	0.78
	69-72	non- Bt		1.96	0.071	0.88	0.049	0.89	0.039	0.89	0.029	0.90	0.008	0.76
	69-71	Bt	Middle	2.03	0.042	0.72	0.033	0.77	0.027	0.78	0.021	0.78	0.005	0.68
	69-72	non-Bt		2.03	0.069	0.77	0.049	0.81	0.039	0.82	0.029	0.80	0.008	0.66
	69-71	Bt	Lower	1.94	0.053	0.88	0.038	0.89	0.029	0.88	0.021	0.84	0.005	0.69
	69-72	non-Bt		1.91	0.064	0.71	0.047	0.80	0.039	0.83	0.030	0.83	0.008	0.55
Snook TX	34-11	HFC Bt	Upper	1.95	0.063	0.96	0.044	0.94	0.035	0.92	0.025	0.88	0.006	0.69
	34-10	HFC non-Bt		1.97	0.064	0.74	0.044	0.81	0.034	0.82	0.025	0.81	0.009	0.69
	34-11	HFC Bt	Middle	1.99	0.067	0.96	0.046	0.94	0.036	0.92	0.025	0.86	0.006	0.68
	34-10	HFC non-Bt		2.04	0.071	0.97	0.048	0.93	0.036	0.90	0.025	0.85	0.006	0.71
	34-11	HFC Bt	Lower	1.92	0.048	0.95	0.044	0.94	0.035	0.92	0.025	0.87	0.007	0.74
	34-10	HFC non-Bt		2.00	0.063	0.85	0.04	0.94	0.033	0.94	0.024	0.90	0.006	0.73

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