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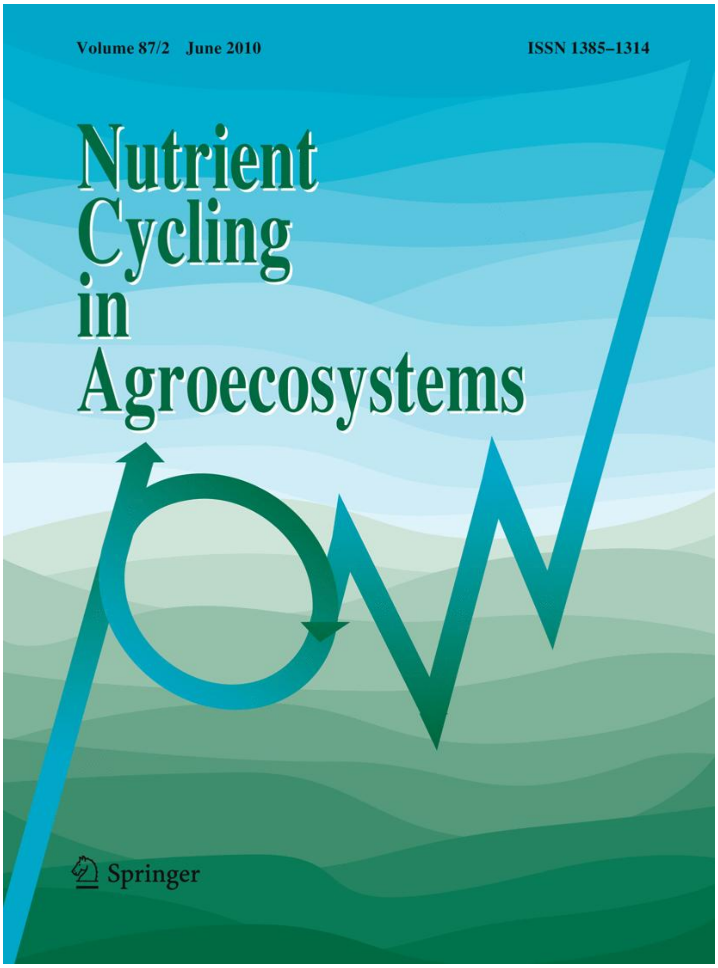

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Crop residue chemistry, decomposition rates, and CO_2 evolution in Bt and non-Bt corn agroecosystems in North America: a review

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Abstract Corn (*Zea mays* L.) is a major cereal crop, with production on more than one-fifth of the agricultural land worldwide. In North America, about 50% of corn acreage is planted with transgenic corn hybrids such as those with the gene from *Bacillus thuringiensis* (Bt) that express the insecticidal crystalline protein (Cry1Ab) for the control of European corn borer (ECB, *Ostrinia nubilalis* Hubner). Widespread production of Bt corn could affect soil organic carbon (SOC) storage in agroecosystems if transgenic corn differs from conventional corn in yield and chemical composition. Generally, the yield of Bt corn is greater than non-Bt corn in years when there is severe infestation of corn insect pests. Some authors report that Bt corn has higher lignin content than non-Bt corn, whereas others found no difference in the chemical composition of near isolines. Residues with higher lignin content are expected to have a slower decomposition rate and release less CO_2 to the atmosphere; however, this is not supported by the literature. A few studies have examined decomposition of Bt corn residues in this context, and the findings to date have been inconclusive, perhaps due to the variety of experimental approaches used to study this question. Generally, the literature supports the view that decomposition rates in Bt corn- and non-Bt corn-amended soils are similar. Whether Bt corn has greater lignin content or slower decomposition rates, the relevant question is whether this will affect the amount of C storage in the soil. A significant gain in SOC requires crop residue inputs with higher lignin content than what is realistically expected from Bt corn residue.

Keywords Bt corn · European corn borer · Lignin content · Residue decomposition · Soil carbon · *Zea mays*

Abbreviations

ADF	Acid detergent fiber
ADL	Acid detergent lignin
Bt	*Bacillus thuringiensis*
Cry protein	Crystal-like protein
ECB	European corn borer
IPCC	Intergovernmental panel on climate change
NDF	Neutral detergent fiber
NPP	Net primary production
SOC	Soil organic carbon
US-EPA	US environmental protection agency

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Introduction

Atmospheric CO_2 concentrations have been increasing exponentially since the Industrial Revolution due to fossil fuel burning, cement production and land use change. The conversion of grasslands and forests to field crop production and deforestation have released about 200 Pg C to the atmosphere since the mid 19th century, with about 90 Pg C from deforestation alone (Reay and Pidwirny 2006). Agricultural soils possess about 128–160 Pg C (Paustian et al. 2000; IPCC 2001) and are perhaps the largest active C reservoir. Agricultural soils may act as a sink or source of CO_2 depending on land management. They can potentially store some of the atmospheric CO_2 fixed by crop plants and hence mitigate greenhouse gas emissions from the agricultural sector.

Soil carbon storage in agroecosystems under corn (*Zea mays*) production has captured our attention for several reasons. Worldwide, more than 20% of agricultural land is planted with hybrids of this adaptable C4 plant (Amos and Walters 2006). In temperate regions, corn plants can achieve a height of more than 2.25 m and an aboveground biomass of 15.9–24.0 Mg ha^{-1} (silage) in a growing season (Jones 2003). An additional 3–5 Mg ha^{-1} of biomass is accumulated in the root system (Prince et al. 2001), which is especially interesting in the context of soil C storage because corn roots are not removed at harvest (Amos and Walters 2006). An example based on a corn agroecosystem in southern Ontario, Canada shows that the crop fixes 10 Mg C ha^{-1} $year^{-1}$ through photosynthesis and loses 3 Mg C ha^{-1} $year^{-1}$ through respiration (Fig. 1) (Lal et al. 1997), thus the net primary production (NPP) would be 7 Mg C ha^{-1} $year^{-1}$. This is consistent with NPP values for corn grown in North America, mostly in the U.S. Midwest, which were calculated to be 7.6 Mg C ha^{-1} $year^{-1}$ using data from Prince et al. (2001). In Fig. 1, grain harvest removes 2.5 Mg C ha^{-1} $year^{-1}$ from the corn agroecosystem and there is a balance between the C entering the soil in crop residues (4.5 Mg C ha^{-1} $year^{-1}$) and CO_2 respired by soil organisms during crop residue decomposition.

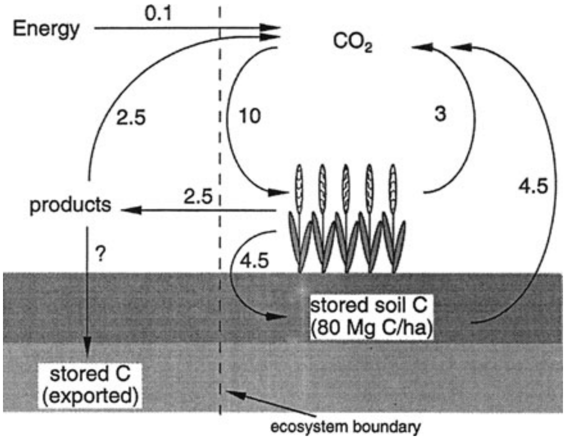

Fig. 1 Approximate annual carbon fluxes in a typical corn agroecosystem in southern Ontario. All fluxes are in Mg carbon ha^{-1} $year^{-1}$ (Lal et al. 1997)

Since the commercial introduction of transgenic crops for field production in 1996, many producers have begun planting transgenic corn hybrids instead of conventional corn hybrids. The first transgenic hybrids were modified at a single locus with genetic material from the bacteria *Bacillus thuringiensis* (Bt) that provided control against Lepidoptera larvae, in particular, European corn borer (ECB, *O. nubilalis*). While Bt corn is still popular, the "stacked" transgenic hybrids that provide resistance to insect attack and tolerance of herbicide damage are favoured in some regions. At present, Bt corn accounts for about 57% of corn production in the US (Economic Research Service/USDA 2008) and 53% of the corn grown in Canada (Canadian Corn Pest Coalition 2007).

It has been suggested that Bt corn differs from non-Bt corn hybrids in yield and chemical composition, which could affect residue decomposition and CO_2 emissions from the soil organic C (SOC) pool. The objectives of this review are to (1) examine the C input from residues in corn agroecosystems, (2) compare the yield and chemical characteristics of residues from Bt corn and non-Bt corn, and (3) evaluate the decomposition rates of Bt corn and non-Bt corn residues.

Carbon input from corn residues

Root residue

Roots interact with the soil environment, assimilating minerals and water from the soil solution and releasing organic materials. They play an important part in C flow in the soil–plant system, since 16–33%

of the C assimilated by plants through photosynthesis is transferred into the soil through the roots (Heal et al. 1997). Corn has a more extensive root system than most other annual crops, thus providing a greater root C input. A three-year field study showed that the post-harvest C input from corn roots was more than double the C input of wheat or soybean roots (Buyanovsky and Wagner 1986).

Root C inputs to the soil come from rhizodeposits and root biomass (dead roots). There is great variation in the reported values of C input through rhizodeposits and root biomass in the literature due to differences in experimental design and study conditions (Amos and Walters 2006) and the difficulty of quantifying rhizodeposits due to their labile nature. While root exudation is probably a major soil C input, there is very little data on flux rates and chemical composition of exudates under field conditions.

Rhizodeposits include root cap cells, mucilage and exudates, a diverse group of substances excreted actively or secreted passively by growing roots. While it is not known precisely how much rhizodeposition occurs under field conditions, values from growth chamber and greenhouse studies provide some insight into the magnitude of C transferred from corn roots to the rhizosphere soil. Molina et al. (2001) reported that rhizodeposits constitute an estimated 24.4% of the C fixed by plants through photosynthesis and that they are rapidly assimilated by microorganisms for metabolic processes (respiration, growth). Buyanovsky and Wagner (1986) estimated that rhizodeposit C constitutes 40% of the total root-derived C (root biomass + rhizodeposits). Rhizodeposits recovered in soil microbial biomass and SOC pools represented 5.2–61.8% with an average of 29% of total root-derived C (root biomass and rhizodeposits) of corn plants at different growth stages grown in the field (two studies) and growth chambers (ten studies) (Amos and Walters 2006). The C contribution of rhizodeposits based on 29% of total biomass and an average corn root biomass of 2.1 Mg ha^{-1} (averaged from field grown corn data at late growth stages based on 75,000 plants ha^{-1} from Amos and Walters 2006) would be 0.6 Mg ha^{-1}.

As for the root biomass remaining in the soil after harvest, it represents an average of 15% of the corn aboveground biomass (Prince et al. 2001). The decomposition of dead root residues by soil microorganisms produces CO_2 and leads to the eventual humification and physical stabilization of C from root biomass. Molina et al. (2001) estimated that root residues account for about 50% of the SOC pool and Johnson et al. (2006) proposed that 1.5–3 times more root C than shoot C is stabilized in the SOC pool, which suggests that root biomass makes a greater contribution to soil C sequestration than aboveground residues. Root biomass has considerable value for SOC storage because of the amount of C contained in these residues and the fact that they are less easily mineralized than rhizodeposits, thus more likely to become chemically or physically stabilized in deeper soil layers (Bolinder et al. 1999).

Shoot residue

For the purpose of this review, the shoot is considered to be the stem and leaves of the corn plant excluding the cob and grain. On average, a whole corn plant at physiological maturity contains 436 kg C per 1,000 kg dry matter, distributed as follows: 26.6% in the leaves, 24.5% in the stem, 32% in the grain, 7% in the roots, and 9.8% in the cob (Latshaw and Miller 1924). There have been many studies that examined the C input and decomposition of aboveground corn residues; an estimated 3–4 Mg C ha^{-1} year^{-1} are added to the soil from aboveground crop residue in corn agroecosystems in the USA (Johnson et al. 2006). About 7.7–20% of the corn shoot residues are retained in the SOC pool in long-term field experiments (Bolinder et al. 1999).

Many producers in North America are planting Bt corn, but the consequence of this activity on SOC pools is not known. High-yielding Bt corn is expected to produce more grain and plant biomass than conventional corn, which could increase the C input from shoot residues left behind at harvest.

Transgenic Bt corn

Bt corn types

Bt corn is a transgenic corn hybrid containing a gene from the naturally occurring gram-positive soil bacteria *B. thuringiensis*. This gene produces a crystal-like protein (Cry protein) that is toxic to many insects that have an alkaline gut and are unable to digest the protein (Schnepf et al. 1998; Saxena and Stotzky

2001a). Different Bt strains produce different Cry proteins that are toxic to specific insects or insect orders. The Cry1Ab is the protein expressed in most Bt corn hybrids against the ECB but some express the Cry1Ac and Cry9C proteins; other Cry proteins from the Bt gene insertion are expressed in other crops (ex: cotton, potato, canola) against their specific pests. Generally, ECB larvae die within two days of ingesting the Bt corn tissue. Expression of the transgene depends on the transformation event used to incorporate the Bt gene into the corn genome. The corn Bt plant pesticides registered with the US Environmental Protection Agency (EPA) are shown in Table 1. The Bt11 and MON810 events produce Bt toxin in all plant parts and are effective against 1st, 2nd, and 3rd generation of ECB larvae. Table 2 shows the amounts of the Cry1Ab protein produced in each of the Bt11 and MON810 corn plant components.

After ECB, corn rootworm (*Diabrotica* spp.) has now become the most destructive and widespread insect pest of corn in North America. Yield and quality losses, harvest time delays, and insecticide costs, in the USA and Canada are substantial (Metcalf 1986; Ostlie 2001). A genetically modified corn with rootworm resistance (CRW-Bt) (MON 863) was released in Canada (DKC42-23) in 2003. The gene encompasses the coleopteran specific insecticidal delta-endotoxin (Cry3Bb) from *B. thuringiensis*. At a clay loam site and under heavy infestation, it was also observed that the CRW-Bt hybrid had a yield advantage of 10–66% compared to the non-Bt control hybrid (Ma et al. 2009). Bt corn hybrids targeting rootworm are being used in Canada and the US (Table 1) but a full discussion on rootworm resistant corn is beyond the scope of this review.

Fate of Cry proteins in the soil and their effect on soil organisms

Cry proteins from Bt transgenic crops are added to the soil through root exudation when the plants are alive and through crop residues when they are incorporated in the soil (Stotzky 2000) and there are concerns that they might have adverse effects on non-target soil organisms, which in turn will affect decomposition and other biological processes in the soil ecosystem. Cry proteins have been shown to bind to clay particles and humic substances (e.g. Tapp and Stotzky 1998; Saxena and Stotzky 2000) and persist in the soil (e.g. Zwahlen et al. 2003) retaining their insecticidal activity (e.g. Tapp and Stotzky 1995) but in general have no persistent negative effects on soil organisms. Readers are referred to a comprehensive review conducted by Icoz and Stotzky (2008) about the fate and effects of Bt crops in soil ecosystems. Their general findings indicate no effect of the Bt toxins on earthworms, woodlouse, collembolans or mites from field experiments and few negative effects on nematodes. Two studies reported fewer mycorrhizal fungi colonization of Bt roots with the 176 Bt insertion event, which is not registered for use at present.

Table 1 Bt corn types classified by gene transformation event. Data from Hagerman (1997), Hyde et al. (1999), US-EPA (2001), Baute (2004), Dow Agrosciences (2007), and Icoz and Stotzky (2008)

Event	Cry protein	Brand name
176	Cry1Ab	KnockOut®—Novartis[a] (field and popcorn)
176	Cry1Ab	NatureGard®—Mycogen[a]
Bt11	Cry1Ab	YieldGard® ® or Attribute™—Northrup King/Novartis Seeds (field and sweet corn)
MON810	Cry1Ab	YieldGard®—Monsanto
TC1507	Cry1F	Herculex1—Dow AgroSciences/Pioneer Hi-Bred
MIR604	mCry3a (modified Cry3a)	SYN-IR604-8 (rootworm)—Syngenta
CBH-351	Cry9C	StarLink™—Aventis Crop Science (corn for feed or industrial uses only) (not approved in Canada)
MON863	Cry3Bb	YieldGard® Rootworm—Monsanto
DAS-59122-7	Cry34Ab1 + Cry35Ab1	Herculex RW (rootworm)—Dow AgroSciences/Pioneer Hi-Bred

[a] EPA registration expired and not renewed

Table 2 Expression of Cry protein in plant tissue, on a fresh weight basis unless otherwise noted (Canadian Food Inspection Agency 1996, 1997, 2007; US-EPA 2001; Dow Agrosciences 2007)

Active ingredient	Leaf (ng mg^{-1})	Root (ng mg^{-1})	Pollen (ng mg^{-1})	Grain (ng mg^{-1})
Cry1Ab (176)	0.44–0.47[a]	0.008	1.14–2.35	<0.005 (kernel)
Cry1Ab (Bt11)	3.3	2.2–37 ng mg^{-1} protein	<0.09 dry wt. pollen	1.4 (kernel)
Cry1Ab (MON810)	7.9–10.3	Not available	0.09	0.19–0.39
Cry1F	111	Not available	136	90
mCry3a	5–26 (dry wt.)	7–25 (dry wt.)	0	0.85 (dry wt. kernel)
Cry9C	44	25.9	0.24	18.6 (kernel)
Cry3Bb1	30–93	3.2–66	30–93	49–86
Cry34Ab1 + Cry35Ab1	67.4 + 43.3	54.9 + 10.4 (R1 stage root)	68.1 + 0.14	45.7 + 1.61

[a] At physiological maturity

European corn borer infestation effects

The infestation of corn with ECB fluctuates from year to year and differs among geographical regions. ECB can produce as many as three generations of larvae per year, with an average of two generations in cooler temperate regions like southern Manitoba, Ontario and Quebec, Canada. Losses from ECB include stalk injury by first and second generation larvae, stalk lodging, ear drop due to second generation larvae, and enhancement of stalk rot in the injured stalks (Willson and Eisley 2001). In addition, ECB injury lowers grain yield because damaged stalks reduce the translocation of photosynthates within the plant (Martin et al. 2004), and the combine harvestor is unable to pick up the ear from the ground, i.e. reduces harvestable yield. Yield loss depends upon the growth stage when the plant is infested (Bode et al. 1990) and on the number of larvae in the stalk. On average, a yield loss of 5.5% from first generation larvae and 2.8% from second generation larvae is expected when the infestation rate is one larva per stalk (Bode et al. 1990), and a combined corn yield loss as high as 12% could occur from first and second generation larvae (Hagerman 1997).

Grain yield losses are not expected to influence the amount of residue that is returned to the soil after harvest, however, injury to stalks could affect the amount of aboveground biomass produced and the rate of crop residue decomposition. The healthy stalks of Bt corn could translocate photosynthates through the plant and increase the C input through rhizodeposition, compared to ECB infested corn. Insect wounding triggers a biochemical cascade that modifies cellular and physiological responses through induced systemic resistance, and could lead to differences in the chemical composition of residues from Bt corn and conventional corn hybrids (Lewis and Yamamoto 1990; Smith et al. 1994). These hypothesized differences between Bt and non-Bt corn would affect decomposition rates and SOC pools in corn agroecosystems of North America. The next sections of this review will provide a critical analysis of findings from studies that evaluated the yield and residue composition of Bt corn and non-Bt corn, as well as the decomposition of residues from these hybrids.

Yield of Bt corn

The yield of Bt and non-Bt corn near-isolines from seven studies conducted at experimental field sites in the USA and Canada is summarized in Table 3. Soil textures ranged from silt loam to clay loam, and a variety of cultivation practices (plant populations, tillage, herbicides, etc.) were used. The mean grain yield from five studies with 18 Bt/non-Bt pairs was 8,829 kg ha^{-1} for Bt corn and 8,367 kg ha^{-1} for non-Bt corn (Folmer et al. 2002; Dillehay et al. 2004; Ma and Subedi 2005; Mungai et al. 2005; Subedi and Ma 2007). The Bt corn produced 1–11% more grain than non-Bt corn, except in the study by Ma and Subedi (2005) where there was 3% less grain from Bt than non-Bt corn (Table 3).

The silage yield/aboveground biomass from six studies ranged from 11,350 to 36,315 kg ha^{-1} for Bt corn and 9,910–39,453 kg ha^{-1} for non-Bt corn (Table 3). Silage yields ranged from 17% lower to as much as 47% higher in agroecosystems with Bt

Table 3 Yield of Bt and non-Bt corn from selected studies in North America. We assumed that yield values were reported on a dry matter basis. Aboveground biomass, plant yield, and silage refer to all aboveground parts, including grain. Calculations for silage/aboveground biomass yield included whole plant data (Jung and Sheaffer 2004) as well as silage and aboveground biomass estimates. The Bt and non-Bt corn yields (average ± SE) from these studies are provided at the end of the table

Study paper	Experimental conditions	Description	Yield range (kg ha^{-1})	Yield average (kg ha^{-1})	% Difference between Bt and non-Bt
Mungai et al. (2005) Missouri, USA	5 Bt and 5 non-Bt isolines no-till planted for 2 years. ECB infestation observed.	Bt grain	4835–8102	6704	
		Non-Bt grain	5239–6704	6075	10.4
Dillehay et al. (2004) Pennsylvania & Maryland, USA	1 Bt, 1 non-Bt isoline, & 1 lead non-Bt hybrid grown in 15 experiments on 2 sites (Pennsylvania & Maryland) over 3 years. ECB infestation observed.	Bt grain	3900–13300	9100	
		Non-Bt grain	4000–13500	8600	5.8
Ma and Subedi (2005) Ottawa, Ontario, Canada	Average of 3 Bt/non-Bt pairs (2000), 6 Bt/non-Bt pairs (2001), 7 Bt/non-Bt pairs (2002) (total hybrids used over years is 7). ECB infestation observed.	Bt grain	4170–9310	6570	
		Non-Bt grain	4350–9250	6780	−3.1
Subedi and Ma (2007) Ottawa, Ontario, Canada	Average of 1 Bt/non-Bt pairs over 2 years. No significant ECB infestation observed.	Bt grain	NA[a]	10050	
		Non-Bt grain	NA	9075	10.7
Folmer et al. (2002) Nevada, USA	Early maturing N4242Bt, N4242 non-Bt, and late maturing N7333Bt, N7333 non-Bt grown without irrigation for silage at one field and N7333Bt, N7333 grown with irrigation for grain at another field. 33-56% ECB infestation in non-Bt corn observed.	Bt grain (early maturing)	NA	8323	
		Non-Bt grain (early maturing)	NA	7658	8.7
		Bt grain (late maturing)	NA	9509	
		Non-Bt grain (late maturing)	NA	8957	6.2
		Bt grain (late maturing, irrigated)	NA	11549	
		Non-Bt grain (late maturing irrigated)	NA	11424	1.1
Folmer et al. (2002) Nevada, USA		Bt silage yield (early maturing)	NA	31608	
		Non-Bt silage yield (early maturing)	NA	27125	16.5
		Bt silage yield (late maturing)	NA	36315	
		Non-Bt silage yield (late maturing)	NA	39453	−8.0
Ma and Subedi (2005) Ottawa, Ontario, Canada	Average of 3 Bt/non-Bt pairs (2000), 6 Bt/non-Bt pairs (2001), 7 Bt/non-Bt pairs (2002) (total hybrids used over years is 7). ECB infestation observed.	Bt aboveground biomass (stalk, leaf, ear)	8010–14100	11350	
		Non-Bt aboveground biomass	7820–14010	11580	−2.0
Subedi and Ma (2007) Ottawa, Ontario, Canada	Average of 1 Bt/non-Bt pairs over 2 years. No significant ECB infestation observed.	Bt aboveground biomass (stalk, leaf, kernel)	NA	18315[b]	
		Non-Bt aboveground biomass	NA	16928[b]	8.2

Table 3 continued

Study paper	Experimental conditions	Description	Yield range (kg ha^{-1})	Yield average (kg ha^{-1})	% Difference between Bt and non-Bt
Motavalli et al. (2004) Missouri, USA (range from 2 years)	5 Bt and 5 non-Bt isolines planted on one site over 2 years. ECB infestation observed.	Bt aboveground biomass	14568–28889	18568	
		Non-Bt aboveground biomass	10864–21728	16123	15.2
Jung and Sheaffer (2004) Minnesota, USA	Six hybrids (3 MON810 & 3 Bt11) and the non-Bt isolines, field grown at four locations. ECB infestation observed.	Bt plant yield (average 2 DKC hybrids over 4 locations)	NA	21225[b]	
		Non-Bt plant yield (average 2 DKC hybrids over 4 locations)	NA	19875[b]	6.8
		Bt plant yield (N3030Bt one location)	NA	17400[b]	
		Non-Bt plant yield (N3030 one location)	NA	21000[b]	−17.1
Fang et al. (2007) Missouri, USA	1 Merschman Bt and non-Bt hybrid pair, field grown. ECB damage observed in non-Bt.	Bt aboveground biomass	11270–17910	14590	
		Non-Bt aboveground biomass	5750–14070	9910	47.2
		Average Bt grain yield		8829 ± 678	
		Average non-Bt grain yield		8367 ± 664	
		Average Bt silage/aboveground biomass yield		21171 ± 3009	
		Average non-Bt silage/aboveground biomass yield		20249 ± 3346	

[a] NA = data not available

[b] Conversion from g/plant to kg/ha based on estimate of 75,000 plant/ha density

corn, compared to non-Bt corn (Folmer et al. 2002; Jung and Sheaffer 2004; Motavalli et al. 2004; Ma and Subedi 2005; Fang et al. 2007; Subedi and Ma 2007). This wide range is partly due to variations in experimental conditions, yield potentials of selected hybrids pairs, and measurements among studies, as well as differences in the ECB infestation in the reviewed studies.

It is generally accepted that yields are greater with Bt corn than non-Bt corn in years with high ECB infestation (more than two larvae per plant) (Dillehay et al. 2004; Ma and Subedi 2005). Variability in the results presented here suggests there were phenotypic and genotypic differences between corn types and hybrids unrelated to the Bt gene effect, an issue which can be overcome by testing a large number of cultivars under the same conditions. It would be informative to compare Bt corn and non-Bt corn with ECB infestation and analyse specific plant components (grain, cobs, stems, leaves and roots) as well as the usual measures of biomass production. At least we need to know the annual input of C in Mg C ha^{-1} year^{-1} of the non-harvested corn components that contribute to the SOC pool.

Soil carbon inputs from Bt corn

Chemical composition of Bt corn

Table 3 shows that the average amount of C returned to the soil from the Bt and non-Bt residue (aboveground biomass minus grain, assuming 40% C content) is 4,937 and 4,753 kg C ha^{-1}, respectively. The decomposition of the non-harvested corn residues (cobs, stems, leaves and roots) by soil microorganisms results in some CO_2 loss via respiration as well as C stabilization within the SOC pool. Decomposition is affected by the physical integrity and the chemical composition of the residue. Lignin and C/N ratio are especially important because lignin is the most recalcitrant component in the plant tissue and C/N ratio exerts an important control on residue decomposition (Cadish and Giller 1997), which is why our focus will be primarily on these measurements.

The lignin, organic C and organic N content of corn residues in seven studies from North America and three studies from Europe is summarized in Table 4. The lignin content of corn residues was most frequently quantified with the Goering and Van Soest gravimetric method (1970), which gives the Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Acid Detergent Lignin (ADL) of plant tissues (Masoero et al. 1999; Rossi et al. 2003; Jung and Sheaffer 2004; Mungai et al. 2005; Fang et al. 2007; Lehman et al. 2008; Tarkalson et al. 2008). Other analytical techniques to quantify lignin content in corn residues included the Klason method (Jung and Sheaffer 2004; Poerschmann et al. 2005), the acetyl bromide (AcBr) method (Saxena and Stotzky 2001b; Jung and Sheaffer 2004; Flores et al. 2005), and off-line and on-line thermochemolysis (Poerschmann et al. 2005). Generally, it is not possible to directly compare the values obtained from one method with another. As Poerschmann et al. (2005) pointed out, the similarity between the AcBr and Klason values reported by Jung and Sheaffer (2004) is unusual and suggests underestimation of the Klason lignin fraction. Corn residues are generally separated into stems, leaves, stem internodes, shoots (stems plus leaves), and in some cases whole plants, roots and cobs. In most experiments, plants were harvested at physiological maturity, so the lignin content of plant components would be representative of residues found in corn agroecosystems.

More data is available on the chemical composition of corn stems and shoots than other plant components (Table 4). In three studies, the lignin content of stems and shoots was statistically greater in Bt corn than in non-Bt isolines (Masoero et al. 1999; Saxena and Stotzky 2001b; Poerschmann et al. 2005). The average lignin content in the corn stalk from eight studies was between 5.1 and 10.8% for Bt corn and from 3.8 to 8.8% for non-Bt corn (Table 4). The lignin content in Bt corn stems was as much as 63.2% greater or 3.8% lower than the non-Bt isoline (Masoero et al. 1999; Saxena and Stotzky 2001b; Rossi et al. 2003; Jung and Sheaffer 2004; Mungai et al. 2005; Poerschmann et al. 2005; Lehman et al. 2008; Tarkalson et al. 2008). Significant differences in lignin content were reported by Saxena and Stotzky (2001b), based on the analysis of 11 Bt hybrids and 10 non-Bt hybrids grown in the field and in a growth chamber. In that study, the lignin content was analyzed by fluorescence microscopy and staining with toluidine blue in addition to the acetyl bromide method. In three studies where Bt corn stems had less lignin than non-Bt corn (Table 4), the lignin

Table 4 Lignin, organic C and total nitrogen content of plant components originating from Bt corn and non-Bt corn from selected studies. Averages (±SE) do not include the stem values (in italics) from Fang et al. (2007), which were already included by Mungai et al. (2005)

Study by	Experimental conditions	Description	Component	Bt corn			Non-Bt corn			% Difference between Bt and non-Bt lignin content
				Lignin (%)	Organic carbon (%)	Total nitrogen (%)	Lignin (%)	Organic carbon (%)	Total nitrogen (%)	
Tarkalson et al. (2008) (Nebraska, USA)	2 Bt hybrids (MON810) and the non-Bt isolines. In the field for 1 year.	ADL lignin (harvested at physiological maturity)	Leaves	7.8	37.9	1.6	8.8	37	1.5	−11.4
			Stems	6.5	43.6	0.8	6.5	43.5	0.7	0.0
			Cobs	5	46.3	0.6	4.6	45.9	0.7	8.7
Jung and Sheaffer (2004) (Minnesota, USA)	Six hybrids (3 MON810 & 3 Bt11) and the non-Bt isolines, field grown at four locations. 23–35% ECB damage in non-Bt hybrids.	ADL lignin (harvested at physiological maturity)	Whole plant	2.2	NA[a]	NA	2.2	NA	NA	0.0
			Stem internode	6.4	NA	NA	6.5	NA	NA	−1.5
Poerschmann et al. (2005) (Aachen, Germany)	2 Bt hybrids (MON810 and 176) and the non-Bt isoline. No ECB infestation.	Off-line thermochemolysis Lignin confirmed with Klason lignin (harvested at BBCH 75 growth stage[b]	Leaves	3.7	NA	NA	3.5	NA	NA	5.7
			Stems	10.8	NA	NA	8.8	NA	NA	22.7
Mungai et al. (2005) (Missouri, USA)	5 Bt the non-Bt isolines field planted for 2 years. ECB infestation observed.	ADL lignin	Stems	6.8	41.9	0.54	7.0	42.3	0.60	−2.9
Lehman et al. (2008) (South Dakota, USA)	2 Bt (one MON810 and 1 stacked) and 1 non-Bt isoline field planted for 2 years. No ECB infestation.	ADL lignin (harvested at full maturity)	Shoots	5.6	42.9	0.78	5.3	42.7	0.77	6.1
Fang et al. (2007) (Missouri, USA)	1 Merschman Bt and non-Bt hybrid pair, field grown. ECB damage observed in non-Bt.	One hybrid (ADL lignin) (stem component included in data of Mungai et al. (2005)	Leaves	3.3	48.9	0.71	2.1	48.3	0.96	57.1
			Stems	*7.8*	*50.6*	*0.52*	*4.3*	*49.8*	*0.66*	*80.2*
			Roots	11.7	43.5	1.2	9.9	41.7	1.15	18.2

Table 4 continued

Study by	Experimental conditions	Description	Component	Bt corn			Non-Bt corn			% Difference between Bt and non-Bt lignin content
				Lignin (%)	Organic carbon (%)	Total nitrogen (%)	Lignin (%)	Organic carbon (%)	Total nitrogen (%)	
Flores et al. (2005) (Long Island, N.Y., USA)	3 Bt (2 field and one sweet corn) and the non-Bt isolines in pots in plant growth room. No ECB infestation.	AcBr lignin (harvested at production of seeds) lignin data is included in Saxena and Stotzky (2001b).	Stems	–	41.8	1.4	–	39.7	1.1	
Masoero et al. (1999) (Northern Italy)	2 Bt and the non-Bt isolines field grown at 3 locations. 50.2% stalk breakage in the non-Bt due to ECB (7% in Bt).	Average of 2 hybrids (ADL lignin)	Stover	6.2	NA	NA	5.8	NA	NA	7.8
Rossi et al. (2003) (Northern Italy)	2 Bt and the non-Bt isolines field grown at 4 locations. Breakage observed in the non-Bt due to ECB.	Average of 2 hybrids (ADL lignin)	Shoots (stover)	5.1	NA	NA	5.3	NA	NA	−3.8
Saxena and Stotzky (2001b)	11 Bt and 10 non-Bt hybrids in growth chamber.	Range (average) of 11 Bt and 10 non-Bt hybrids in growth chamber (AcBr lignin)	Stems	7	NA	NA	4.9	NA	NA	42.9
Saxena and Stotzky (2001b) (New York, USA)	8 Bt and 7 non-Bt hybrids in field.	Range (average) of 8 Bt and 7 non-Bt hybrids in field (AcBr lignin)	Stems	6.2	NA	NA	3.8	NA	NA	63.2
		Average of all values		6.3 (±0.6)[b]	43.4 (±1.1)	1.0 (±0.1)	5.7 (±0.6)	42.6 (±1.2)	0.9 (±0.1)	
		Average values for stems and shoots		6.7 (±0.5)	42.6 (±0.4)	0.9 (±0.2)	6.0 (±0. 5)	42.1 (±0.8)	0.8 (±0.1)	
		Average values for leaves		4.9 (±1.4)	43.4 (±5.5)	1.2 (±0.4)	4.8 (±2.0)	42.7 (±5.6)	1.2 (±0.3)	

[a] NA = data not available

[b] Cob content milky, about 40% dry matter http://en.wikipedia.org/wiki/BBCH-scale_(maize)

content did not differ between Bt and non-Bt corn stems (Rossi et al. 2003; Jung and Sheaffer 2004; Mungai et al. 2005).

On average, the lignin content in leaves was 4.9% for Bt corn and 4.8% for non-Bt (Poerschmann et al. 2005; Fang et al. 2007; Tarkalson et al. 2008). Single values for cobs and roots are included in Table 4. The nutrient content of corn stems and leaves, averaged from five studies, was 42.8% C and 1.0% N for Bt corn, with 42.3% C and 0.9% N in non-Bt corn (Table 4).

What if Bt corn, or any corn type, has an elevated lignin content? Would that result in an increase in the amount of stable C in the soil? In theory, we can estimate the transformation of plant lignin into stable SOC from a simple decomposition model. For the sake of argument, we hypothesized that Bt corn has an elevated lignin content, compared to non-Bt corn, and tested the impact this might have on residue decomposition and C storage in the soil. Decomposition can be described by an exponential first order equation consisting of two litter pools, one that decomposes rapidly and one that decomposes slowly (Johnson et al. 2007; Bahri et al. 2008). In this example, we considered decomposition as the loss of lignin, rather than the mass loss of litter where:

$$L_t = L_1 \exp(-k_1 t) + L_2 \exp(-k_2 t) \tag{1}$$

L_t is the total lignin (in percent) remaining after time t (days), L_2 is the slow decomposing pool, which is equated with the lignin (in percent) decomposing at the slow constant rate of K_2 (% d^{-1}), and L_1 is 100-L_2, which represents the non-lignin components that decompose rapidly at the constant rate of K_1. The values for the constants K_1 and K_2 (Table 5) are based on the decomposition rate of corn stems from the study of Johnson et al. (2007), determined from a 498-day decomposition experiment.

Two scenarios representing the range of differences between the lignin content of Bt and non-Bt corn in Table 4 were tested. The Bt lignin content was assumed to be 6 or 60% greater than non-Bt, which was assigned a value of 6% (average lignin content in non-Bt stems, Table 4). The results in Table 5 show that after 200 days of decomposition, the amount of lignin in the undecomposed residue was 7% greater in Bt corn residue than non-Bt corn when the Bt corn contains 6% more lignin. If these results can be directly extrapolated to the field scale, an agroecosystem where 10,000 kg ha^{-1} year^{-1} of residue is returned to the soil (50% of the 20,000 kg ha^{-1} of non-Bt aboveground biomass from Table 3), would have up to 38 kg ha^{-1} year^{-1} more lignin remaining from Bt corn after 200 days of decomposition. Since this residue is made up mainly of undecomposed lignin, we propose that it is likely

Table 5 Amounts of lignin remaining after 200 days of decomposition under two scenarios in which Bt corn had greater lignin content than non-Bt corn, calculated from the double exponential decomposition model (Johnson et al. 2007; Bahri et al. 2008). The amount of corn residue returned to the soil is assumed to be 50% of the aboveground biomass = 10,000 kg ha^{-1} year^{-1}

	Time (days)	L_t^a (%)	L_1 exp(-k_1)^b (%)	L_2 exp(-k_2)^c (%)	Amount lignin remaining (kg ha^{-1} year^{-1})	Difference between amount lignin remaining compared to non-Bt residue
Average lignin of non-Bt corn stems	1	94.701	88.703	5.998		
	200	5.682	0.001	5.681	568	–
Scenario 1 lignin is 6% greater in Bt vs. non-Bt corn	1	94.724	88.326	6.398		
	200	6.061	0.001	6.060	606	38
Scenario 2 lignin is 60% greater in Bt vs. non-Bt corn	1	94.903	85.306	9.597		
	200	9.091	0.001	9.090	909	341

[a] Total lignin remaining after 200 days of decomposition

[b] Fast decomposing non-lignin fraction

[c] Slow decomposing lignin fraction

$k_1 = 0.06\%$ d^{-1}, $k_2 = 0.000273\%$ d^{-1}

to be stabilized and become part of the slow turnover SOC pool. These calculations show that a meaningful increase in the amount of stable SOC requires the input of corn residues having 6% more lignin content than conventional corn residues, a scenario that seems very unlikely.

Lignin content of corn

The above example is based on generalized assumptions on the lignin content of corn. However, lignin is a highly heterogeneous compound, randomly formed with variable subunit composition and intermolecular linkages (Lewis and Yamamoto 1990; Campbell and Sederoff 1996). Lignin content varies among plant species and between plant cells, meaning that plant components could have different lignin contents and thus decompose at different rates. The data presented in Table 4 shows that the lignin content of corn plants was greatest in roots (one study), followed by stems, cobs (one study) and leaves.

The lignin composition and content in plant cells is regulated by enzymes in the biosynthetic pathway and gene expression. Environmental stress affects lignin formation and deposition in vascular tissues (Campbell and Sederoff 1996), as does wounding, such as that caused by feeding insects (Lewis and Yamamoto 1990; Smith et al. 1994). Furthermore, wound-induced lignin deposition may cause lignification of the whole cell, in contrast to normal lignification, which occurs only in the cell wall (Lewis and Yamamoto 1990). Although ECB damage was reported in some studies reviewed in Table 4, there was no analysis of the lignin deposition in relation to ECB wounding. We suspect that corn plants infested with ECB, causing tunnelling and partial decomposition of the stalk, may differ in lignin content from uninfested corn plants but there has been no literature published to support this yet.

Qualitative measurement of molecular lignin composition

Cupric oxide oxidation and degradative thermal methods among others release the vanillyl (V), syringyl (S), and cinnamyl (C) phenols from the lignin molecules, which can then be measured by the gas chromatography-mass spectrometry (GC–MS). The sum of the three phenols known as VSC has been used as an indicator of total lignin but is not a quantitative measure of lignin especially when using the CuO oxidation method. In CuO oxidation, the lignin molecule is not completely depolymerised (Otto and Simpson 2006) and there are interferences from side-chain reactions (Poerschmann et al. 2005). In addition, the ratio of acid to aldehyde of vanillyl and syringyl is used as an indicator of the degree of decomposition of organic matter, as this ratio increases as decomposition progresses (Poerschmann et al. 2005; Otto and Simpson 2006). Poerschmann et al. (2005) used these methods to study the difference between Bt and non-Bt corn lignin at the molecular level. In their study, two pairs of Bt and non-Bt corn hybrids, Novelis (MON810 event) and its isoline Nobilis, and Valmont (176 event) and its near-isoline Prelude, were field grown in Germany where no ECB infestation was observed. The Bt components had significantly higher lignin contents than the non-Bt components; the average ratio of lignin content in Bt/non-Bt stems (between the 4th and 5th internodes) and leaves was 1.23 and 1.18, respectively. The increase in lignin was mainly due to an increase in the guaiacyl-type lignin (made from vanillyl precursors) and to a lesser extent to an increase in the hydroxyphenyl-type lignin (made from cinnamyl precursors). This was true for both stems and leaves, but was more pronounced in stems. The guaiacyl-type lignin monomers are considered more recalcitrant than the other two lignin monomers (syringyl and cinnamyl derived), which reinforces the belief that Bt corn residue, especially the stems, are stronger and more resistant to degradation.

Decomposition of Bt corn residues

Corn residues constitute a soil C input, and plant components with high lignin contents are expected to have an extended residence time in the soil and therefore contribute to soil C sequestration (Zibilske and Materon 2005). The reviewed studies suggest that there may be more corn residues left in agroecosystems with Bt corn than non-Bt corn (Table 3). In addition, the lignin content tends to be greater in the stems and shoots of Bt corn (Table 4). These factors are expected to slow decomposition rates and increase the stabilization of the C inputs from transgenic corn hybrids (Saxena and Stotzky 2001b; Hopkins and Gregorich 2003). Yet, the experimental evidence does not support this hypothesis unequivocally. Three decomposition studies with controlled-environment conditions showed lower CO_2 production in Bt compared to non-Bt corn residue amended soils (Dinel et al. 2003; Castaldini et al. 2005; Flores et al. 2005). In contrast, field decomposition litterbag studies showed either no difference in mass loss between Bt and non-Bt corn residues buried in the field (Lehman et al. 2008; Tarkalson et al. 2008) or faster decomposition of Bt leaves compared to non-Bt leaves during early decomposition, after which the two hybrids levelled off at the end of the study (Zwahlen et al. 2007).

In their laboratory experiment, Flores et al. (2005) reported significantly lower CO_2 production (20–39% less decomposition during a 32–42 day incubation period) from soils amended with Bt residue than non-Bt residues of a corresponding crop. They tested transgenic and unmodified cultivars of corn, rice, potato, cotton, canola and tobacco. The lower CO_2 production was not related to differences in C:N ratio or lignin content, or of the inhibition of the activity of soil microbiota (Table 6). Similarly, Castaldini et al. (2005) found 10% less CO_2 respiration from soil incubated with Bt corn residue, but the comparison was done with one Bt11, one 176 Bt and one non-Bt variety that was not an isoline or near isoline to either of the Bt varieties. It is possible that the results were influenced by corn varieties selection. Dinel et al. (2003) incubated a silty clay loam soil from Sainte-Barbe in Quebec, Canada, with Bt shoots (Pioneer 38W36) and with non-Bt shoots (Pioneer 3893) and measured 30.5% more cumulative CO_2–C production from the non-Bt amended soil after 51 days of incubation.

In contrast, Hopkins and Gregorich (2003) reported no difference between CO_2 evolutions in Bt versus non-Bt corn residue amended soils. The plant residues used for this study were the vegetative shoots of corn harvested at the 6-leaf stage (Hopkins and Gregorich 2003), while Castaldini et al. (2005) used 10–12-week-old corn shoots as residue and Flores et al. (2005) used the aboveground residues from plants that had achieved physiological maturity. Some of the difference in the results may arise from the phenological stage at which corn residues were collected. Fang et al. (2007) obtained root, stem, and leaf residue from Bt and non-Bt corn, and plant parts were added separately to laboratory microcosms containing soils with different textures. The Bt factor had no effect on CO_2 efflux even though the Bt components had higher lignin and C:N than the non-Bt components. The residue component and soil texture significantly affected CO_2 efflux.

In the field, Tarkalson et al. (2008) studied decomposition of two Bt corn hybrid residues and their isolines in litterbags and reported that the decomposition rates for leaves, cobs, and stalks were similar for Bt and non-Bt hybrids. Lehman et al. (2008) also used the litter bag technique in the field, but found no differences in decomposition of Bt and non-Bt corn residue even though the Bt corn tissue had higher lignin content. They commented on the fact that there was no insect stress during the experiment and that the presence of such stress might result in weaker non-Bt stalks that are less resistant to decay. It was also noted that the authors were unable to measure mass loss after about one year of the litter burial, which includes the last 20% of the litter residue (presumably the more recalcitrant portion). The reason for this could be due to the increase in the soil to litter ratio which made differences in mass difficult to detect.

Lehman et al. (2008) measured the mechanical strength of the chopped corn tissue and found that all

Table 6 Carbon dioxide (CO_2) evolution from soils amended with 0.5% (by weight) residue from Bt corn and non-Bt near isolines (data from Flores et al. 2005)

Bt isoline	Residue type and % added (w/w, dry tissue)	CO_2 evolution (mg C/100 g soil)	C:N (leaf)	C:N (stem)	Non-Bt isoline	CO_2 evolution (mg C/100 g soil)	C:N (leaf)	C:N (stem)
NK4640Bt	Stem + leaf at 0.5%	52.3	79	149.9	NK4640	73.1	80.3	171.8
966	Stem + leaf at 0.5%	55.2	–	–	Prime Plus	76.3	–	–
DK647Bty	Stem + leaf at 0.5%	58.3	–	–	DK647	75.3	–	–
NK6800Bt	Leaves at 0.5%	91	38.4	26	NK6800	108	26.6	17.5
NK6800Bt	Stem at 0.5%	112	38.4	26	NK6800	181	26.6	17.5

Bt types tended to have stronger, more physically resistant residues, although not significantly different from non-Bt types. They suggested that similar analysis be conducted on the un-chopped tissue to see if the chopping had an effect on strength and consequently on decomposition in the field. This point suggests that the tissue size and preparation for decomposition/incubation studies might have an effect on the results.

Results from the above reviewed laboratory and field studies suggest that decomposition rates and CO_2 evolution from Bt corn residue-amended soils are often similar to those amended with non-Bt residue. This is true even where the Bt corn had higher content of the more recalcitrant cell constituents such as cellulose and lignin (Flores et al. 2005; Fang et al. 2007; Lehman et al. 2008). It is noted that almost all the studies focused on the decomposition of aboveground crop residues (leaves, stems and cobs), and root decomposition was seldom examined.

Decomposition of corn components

Tarkalson et al. (2008) found that corn components differed significantly in their decomposition. After 23 months in the field, the leaves, stalks, and cobs retained 5.5, 17.7, and 38.6% of their total C content, indicating that cobs were the slowest to decompose. Table 7 shows the average chemical composition of these plant components. The soluble fraction appears to control the decomposition rate in addition to the C:N ratio of the plant components, since leaves, having the highest percentage of the soluble fraction and the lowest C:N ratio, decomposed rapidly even though they contained higher lignin than stalks or cobs (Table 7). In the Merschman hybrids M-0012Bt and M-00110, Fang et al. (2007) reported that stems had the highest C content and C:N ratio followed by leaves then roots. Decomposition was not correlated to the C:N ratio, as leaves decomposed more quickly than stems and roots, suggesting that leaf tissues contained more soluble than structural carbon than stems and roots. They also noted that after 73 days of incubation, the factor that most significantly affected cumulative decomposition was the soil texture. In the silty clay soil, Bt roots, which had the highest lignin content among all Bt and non-Bt components, decomposed slower than Bt leaves and Bt stems whereas the non-Bt roots had a faster decomposition rate. This may be related to the presence of soil microbial communities capable of degrading lignin and other resistant compounds in roots. Filamentous fungi (mainly basidiomycetes) are key organisms for lignin degradation (Hammel 1997). As discussed earlier, the decomposition of roots from Bt corn has not received much attention, although roots constitute a considerable soil C input and may be more slowly decomposed than other corn residues. Studies are needed to consider the decomposition of Bt corn roots in the context of SOC dynamics and soil C sequestration.

Table 7 Chemical composition of the fiber fraction (decreasing order) in leaves, stalks, and cobs of corn hybrids (average of four hybrids). Data from Tarkalson et al. (2008)

Leaves	Stalks	Cobs
Soluble fraction (40.3%)	Cellulose (36.9%)	Hemicellulose (42.8%)
Hemicellulose (28.6%)	Soluble fraction (34.5%)	Cellulose (35.1%)
Cellulose (23.2%)	Hemicellulose (21.8%)	Soluble fraction (17.3%)
Lignin (8.0%)	Lignin (6.5%)	Lignin (4.8%)

Conclusions

Given that Bt corn production is increasing globally and accounts for about 50% or more of the corn production in North America if stacked hybrids (e.g., Bt plus Roundup Ready genes) are included, it is worth examining the effects of this crop on SOC dynamics. There is evidence that higher Bt corn yields produce more residues than non-Bt corn, constituting a greater C input to agroecosystems. Some studies suggested that the insertion of the Bt transgene into the corn genome could affect the lignin content of Bt corn, however, it should be noted that the Bt transgene does not affect the biosynthetic pathway of lignin production in the plant (Jung and Sheaffer 2004) and should not affect the amounts of lignin produced. Since only three out of the seven reviewed studies had significantly greater lignin content in the Bt corn tissue, it could be argued that the greater lignin is due to difference in the phenotypic characteristics of the selected hybrids. In most of the studies the Bt and non-Bt hybrids were not isolines but rather near-isolines, which means that they are not exactly the same and may vary in their crop heat units requirement, among other traits. In addition, there appears to be no difference in the decomposition rates of Bt and non-Bt corn residues even when Bt residues exhibited greater lignin contents, and no lasting adverse effects on soil decomposers has been noted. This suggests that the impact of Bt corn production on the soil ecosystem is minimal, though this may be more appropriately stated for soils that are not continuously under Bt crop production for the long term.

Finally, corn plants for many experiments were grown in greenhouses or growth chambers without the introduction of the ECB larvae, which are common in the field. The response of corn to stress conditions and injury of the vascular tissue from the ECB could lead to differences in chemical composition and should be considered as a factor when conducting experiments that compare Bt corn and non-Bt corn.

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