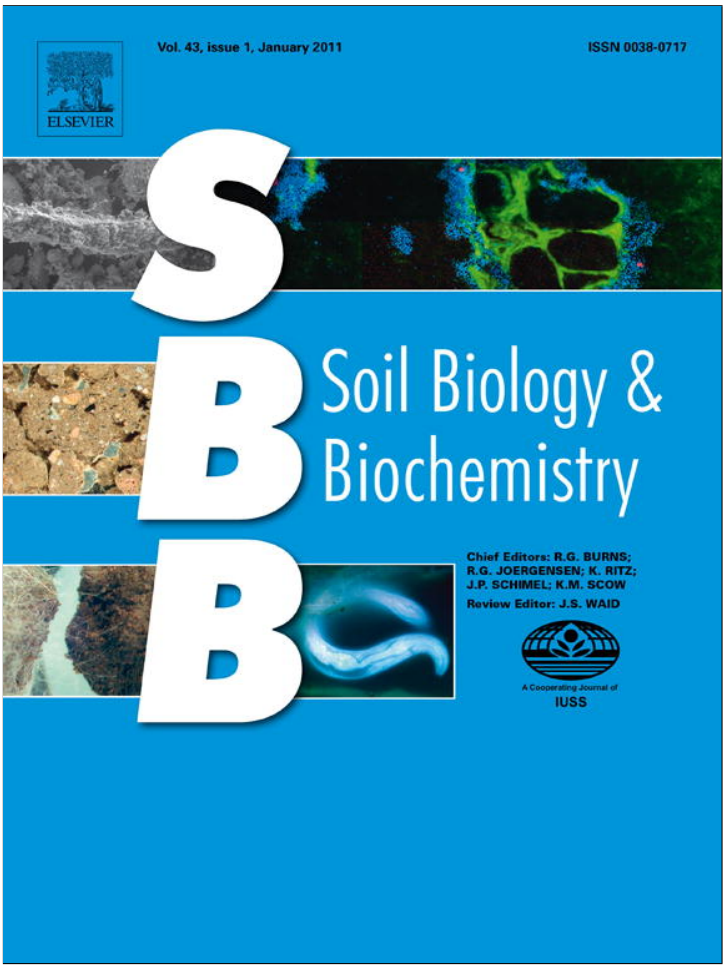

Soil Biology & Biochemistry 43 (2011) 63–69

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Plant lignin and nitrogen contents control carbon dioxide production and nitrogen mineralization in soils incubated with Bt and non-Bt corn residues

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ARTICLE INFO

Article history:
Received 15 April 2010
Received in revised form
15 August 2010
Accepted 13 September 2010
Available online 27 September 2010

Keywords:
Bacillus thuringiensis
Corn
Carbon dioxide production
Incubation
Lignin
Lignin-derived phenols
Nitrogen mineralization

ABSTRACT

Bt (*Bacillus thuringiensis*) corn is reported to produce lignin-rich residues, compared to non-Bt (NBt) corn, suggesting it is more resistant to decomposition. As the Bt gene is expressed selectively in stem and leaf tissue, it could affect lignin distribution in corn, which naturally has greater lignin content in roots than in stems and leaves. Our objective was to evaluate the effects of corn plant components, the Bt gene and elevated-lignin inputs on decomposition. Roots, stems and leaves from Bt corn and NBt corn isolines enriched with ^{13}C and ^{15}N were finely ground and mixed separately with soil, then incubated at 20 °C for 36 weeks. The effect of elevated lignin on decomposition was tested by adding a commercial lignin source (indulin lignin) to half of the samples. In addition to weekly CO_2 analysis and regular measurement of N mineralization, the degree of lignin degradation was evaluated at 1 and 36 weeks from the acid to aldehyde ratio (Ad/Al) of vanillyl and syringyl lignin-derived phenols. The CO_2 production and N mineralization was lower in root-amended soils than stem- and leaf-amended soils. The Bt genetic modification increased CO_2 production from stem-amended soils ($P < 0.05$) and decreased N mineralization in root-amended soils. The ^{13}C and ^{15}N results also showed more residue-C and -N retained in soils mixed with NBt stem residues. After 36 weeks leaf- and stem-amended soils with indulin lignin had a lower Ad/Al ratio and were less degraded than soils without exogenous lignin. In conclusion, plant lignin and nitrogen contents were good predictors of CO_2 production and N mineralization potential. Corn roots decomposed more slowly than aboveground components emphasizing the importance of recalcitrant root residues in sustaining the organic matter content of soil.

1. Introduction

The use of Bt (*Bacillus thuringiensis*) corn (*Zea mays*) to avoid damage from the European corn borer (*Ostrinia nubilalis*) has been increasing since its commercialization in 1996. In 2009, Bt corn hybrids were planted on more than 50% of land under corn in North America (ERS-USDA, 2009). There have been reports that Bt corn differs chemically from corn hybrids without the Bt gene (NBt) and some farmers state that Bt corn residues are tougher than NBt residues and decompose more slowly (Lehman et al., 2008). Such differences could affect soil microbial activity and residue decay, altering N mineralization and CO_2 emitted from agroecosystems where Bt corn is grown.

Lignin content of residue is one of the main factors affecting decomposition due to the recalcitrance of this complex molecule and its resistance to degradation by soil microorganisms and extracellular enzymes (Austin and Ballare, 2010; Cadisch and Giller, 1997; Melillo et al., 1982). Residues with high lignin content are expected to decompose more slowly, and persist longer in soils than residues with low lignin content. Studies comparing the lignin content of Bt and NBt corn are contradictory; some report that lignin content is higher in Bt residues than NBt residues (Poerschmann et al., 2005; Saxena and Stotzky, 2001), others find little effect (Jung and Sheaffer, 2004; Lehman et al., 2008, 2010; Masoero et al., 1999; Mungai et al., 2005; Tarkalson et al., 2008). If lignin content is affected by the Bt gene, which is expressed selectively in leaf and stem tissue, then the extra lignin might not be distributed uniformly in the plant. In addition, lignin content of plants varies naturally, with greater lignin content in roots and stems than in leaves (Abiven et al., 2005; Mungai et al., 2005), so lignin content of different plant parts needs to be considered in residue decomposition experiments (Abiven et al., 2005).

If some Bt hybrids have greater lignin contents than NBt isolines, this would be expected to slow the decomposition of Bt corn

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0038-0717/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2010.09.012

residues, as shown in some laboratory studies (Castaldini et al., 2005; Dinel et al., 2003; Flores et al., 2005) that found lower CO_2 production from soils amended with Bt corn residue, but other authors reported no differences in CO_2 production from soils receiving Bt vs. NBt corn residues in the field (Lehman et al., 2008; Tarkalson et al., 2008) and laboratory (Fang et al., 2007; Hopkins and Gregorich, 2003). Even when the Bt corn residue had greater lignin and lignin:N ratio (Fang et al., 2007), implying lower decomposability, there was no difference between the two residue types. A silt-loam soil amended with NBt corn roots (Merschman-00110) had 2.7 times more N mineralization than soil mixed with Bt roots (M-00112Bt), but this was not observed when roots were incubated in a silty clay or sandy loam soil (Mungai et al., 2005). In addition, the Bt gene did not affect N mineralization from leaves and stems in any soil in this incubation experiment (Mungai et al., 2005).

As the difference in the lignin content of mixed residues from Bt and NBt corn may be too slight to have an effect on decomposition, it would be informative to compare CO_2 production and N mineralization of corn components, namely roots, stems and leaves. Reports from experiments comparing the decomposition of Bt corn, which had 18–80% more lignin than the NBt corn (Fang et al., 2007), showed no difference in CO_2 production whereas lower CO_2 production was reported when the Bt corn had 90% more lignin than the NBt hybrids (Flores et al., 2005). Lignin content of the substrate has a direct effect on decomposition rates and therefore, to be able to test the effect of elevated-lignin contents on decomposition rates it would be useful to add an exogenous lignin source to some treatments for comparison.

The types of phenylpropanoid monomers that make up lignin molecules also affect the recalcitrance of residues. Lignin is formed from the radical coupling of three alcohol monomers, *p*-coumaryl, coniferyl, and sinapyl alcohols incorporated into the macromolecule as the vanillyl (V), syringyl (S), and cinnamyl (C) phenols and their derivatives (Otto and Simpson, 2006). The CuO oxidation method combined with gas chromatograph–mass spectrometry (GC–MS) is used to measure these lignin-derived phenols. Lignin residues that contain more vanillin are more difficult to degrade because vanillin is derived from guaiacyl phenylpropanoid which has a free C_5 position on the aromatic ring, producing more condensed lignin molecules. Brown-rot and white-rot fungi have been shown to selectively degrade syringyl phenols over vanillin phenols (Boerjan et al., 2003; Christiernin et al., 2009; Eckardt, 2002; Hedges et al., 1985), which are considered to be the most recalcitrant of the lignin monomers (Feng and Simpson, 2008). Therefore, plant material with a high vanillyl to syringyl ratio (V:S) can be expected to be more resistant to degradation. The acid to aldehyde ratios (Ad/Al) of vanillin and syringyl can also be used as an indicator of the degree biodegradation or diagenetic alteration of lignin by microorganisms specifically white-rot fungi; as biodegradation proceeds, the monomers are transformed from aldehyde forms to their acid forms, which results in an increase in the Ad/Al ratio (Hedges et al., 1988; Loh et al., 2008; Opsahl and Benner, 1995; Otto and Simpson, 2006; Poerschmann et al., 2005). Poerschmann et al. (2005) reported that total lignin content in stems and leaves was greater in two Bt corn lines than in NBt lines. This was attributed to an increase in guaiacyl-type lignin (specifically, *trans*-3-(3,4-Dimethoxyphenyl)-3-propenoic acid ME) in the Bt corn lines, evaluated by thermochemolysis and CuO oxidation methods. However, the Ad/Al ratio implies that Bt corn stems are more susceptible to oxidation than NBt stems, which suggests that molecular-level transformations of lignin-derived organic matter occur during the decomposition perhaps explaining CO_2 production and the eventual stabilization of plant-derived C in soils.

The first objective of this study was to compare the decomposition of corn roots, stems and leaves with the expectation that roots would decompose at slower rates due to their elevated-lignin content compared to other plant components. The second objective was to determine if residue decomposition differs between Bt corn and NBt corn; the hypothesis is that CO_2 production would be similar between Bt and NBt since the hybrids used in this experiment had a similar chemical composition. We were also interested in assessing the effect of elevated-lignin content of soil–residue mixtures on decomposition, which was tested by the addition of an exogenous lignin source to simulate elevated-lignin content in the added substrate. It is expected that the addition of exogenous lignin would hinder decomposition from those treatments. Correlations between decomposition (CO_2 production) and chemical attributes of corn residues (lignin and N contents, C:N and lignin:N ratios), as well as changes in the Ad/Al ratios of lignin-derived phenols from Bt and NBt corn residue during decomposition were also measured.

2. Materials and methods

2.1. Soil and corn litter

Soil was collected in September 2007 from the 0–20 cm layer of a long-term corn experiment at the Macdonald Research Farm, Ste. Anne de Bellevue, Quebec, Canada (45°30′N, 73°35′W). The soil was a Dystric Gleysol (815 g sand kg^{-1}, 96 g clay kg^{-1}, with a pH of 6.0, 17.6 g organic C kg^{-1} and 1.6 g kg^{-1} N). The soil was air-dried and passed through a 2 mm mesh sieve. A Bt corn (*Z. mays* L.) hybrid (DKC 38-33, MON810 Bt insertion event) and a NBt near-isoline (DKC 38-32) were grown in this sandy loam soil in pots in the greenhouse and enriched with the ^{15}N and ^{13}C according to the method of Bromand et al. (2001). Briefly, this involved weekly pulse-labeling with ^{13}C–CO_2 beginning at the V2 growth stage and adding ^{15}N–KNO_3 fertilizer after each pulse-labeling event. The resulting ^{13}C and ^{15}N enrichment in the corn tissue was above the enrichment of the background soil and corn tissue (Table 1). Corn was harvested at the V9–V10 growth stage (Ritchie et al., 1986) and separated into leaves, stems, and roots. Corn components were dried at 50 °C for 24 h and ground to pass through a 1 mm mesh sieve. The isotope enrichment levels, C and N, and fiber contents of soil, corn and indulin lignin are shown in Table 1.

2.2. Aerobic soil incubation

The experiment was a complete factorial design, with three plant components (roots, stems and leaves) from two near-isolines (Bt and NBt corn), added to soil, from the same site where the corn was grown, with and without indulin lignin (Sigma Chemical Co., St. Louis, MO, 1-6384), for a total of 12 experimental treatments. Four replicates of each treatment were prepared for each of 10 sampling dates (1, 2, 4, 8, 12, 16, 20, 24, 30 and 36 weeks after the beginning of the study) to permit destructive sampling, for a total of 480 jars. Each replicate consisted of 50 g of air-dried soil placed in an acid-washed 120 cm^3 plastic vial with 0.5 g of ground corn tissue. Plastic vials that received the lignin treatment were amended with 0.1 g of indulin lignin (+L). The contents of each vial were mixed thoroughly, moistened to 40% water-filled pore space, and placed inside 1-L Mason jars, along with 10 mL distilled water to maintain soil humidity. Jars were capped with an air-tight lid, incubated in the dark at 20 °C, and lids were removed to aerate the jar for 15 min every week. Air-tight rubber septa were fitted into the lids of the replicates designated for weekly CO_2 sampling. The gas samples were injected into pre-evacuated 12 mL exetainers (Labco, Wycombe, UK) with an extra 60 mil teflon–silicone septa (National Scientific, Rockwood, TN, USA) containing a small amount

Table 1
Organic C, total N, $\delta^{13}C$, $\delta^{15}N$, and lignin contents of the incubation soil, indulin lignin and corn residues.

Sample	Organic C (g kg^{-1})	$\delta^{13}C$	Total N (g kg^{-1})	$\delta^{15}N$	Lignin (g kg^{-1})	C:N	Lignin:N
Soil	16.4	−24.0	1.20	14.12	n.d.*	13.7	n.d.
Unlabeled corn tissue (average of shoots and roots)	n.d.	−12.3	n.d.	1140	n.d.	n.d.	n.d.
Indulin lignin	567	−27.1	7.40	137.5	1000**	76.6	135
Bt Leaves	452	9.80	35.5	4386	28.5	12.7	0.80
NBt Leaves	431	16.1	26.2	3882	35.9	16.5	1.37
Bt Stems	401	2.52	30.8	5721	36.2	13.0	1.18
NBt Stems	412	7.25	15.9	5131	34.6	25.9	2.18
Bt Roots	245	1.79	14.8	4138	54.2	16.6	3.66
NBt Roots	383	10.1	17.8	4288	69.6	21.5	3.91

* n.d. = not determined.
** Assumed but not measured.

of magnesium perchlorate to absorb moisture, for short-term storage. The CO_2 concentration was measured by thermal conductivity detector using a gas chromatograph (Hewlett–Packard 5890 Series II, Hewlett–Packard Company, Avondale, PA, USA) equipped with a Porapak Q column (ethylvinylbenzene and divinylbenzene copolymer beads; 80–100 mesh; length, 25 m; internal diameter, 0.20 mm; Supelco 20331). The carrier gas was helium (50 mL min^{-1}). Oven and detector temperatures were 120 °C and 250 °C, respectively.

CO_2 gas sampling was continued until week 20 at which time CO_2 production had stabilized. The CO_2 value was converted from volume to mass units following the ideal gas equation (Livingston and Hutchinson, 1995):

$$_{sample}CO_2 - C = \frac{C_m \times M \times P}{R \times T} \quad (1)$$

where $_{sample}CO_2$–C is the amount of C in the sample (in mg L^{-1}), C_m is the measured CO_2 (in μL L^{-1}), M is the atomic weight of C (12 g C mol^{-1} CO_2^{-1}), P is the atmospheric pressure (1 atm), R is the universal gas constant (82.06 atm mL mol^{-1} °K^{-1}), and T is the room temperature (298 K). Then, the amount of CO_2 produced (g CO_2–C kg^{-1} soil) was calculated as follows:

$$g\ CO_2 - C\ kg^{-1}\ soil = \frac{_{sample}CO_2 - C \times V}{W} \quad (2)$$

where V is the volume of the headspace in the jar (0.96 l) and W is the weight of the soil used in the incubation (50 g).

On the designated weeks, vials were removed for destructive sampling, the amended soils were thoroughly mixed, and a 5 g sub-sample was immediately extracted with 2 M KCl solution (Maynard et al., 2008) for NH_4–N and NO_3–N analysis with a Lachat Quik Chem Flow Injector Autoanalyzer (Lachat Instruments, Milwaukee, WI 53218, USA). An additional 5 g sub-sample was taken for moisture determination (dried at 50 °C) and kept for ^{13}C and ^{15}N measurements. The plastic vial was sealed with a screw-on lid and the remaining soil was stored at −15 °C for CuO oxidation analysis.

The amount of mineral N (NH_4–N plus NO_3–N) produced during the 36 week incubation was used to calculate the mineralization rate constant (*k*, in $week^{-1}$) by non-linear regression analysis according to the following equation: $N_0 = N_{min}(1 - e^{-kt})$, where N_0 is the cumulative N mineralized (mg N kg^{-1}) after time t (in weeks) and N_{min} is the potentially mineralizable N (mg N kg^{-1}) under optimum temperature and moisture (Curtin and Campbell, 2008).

2.3. Lignin-derived phenol analysis

CuO oxidation was carried out on pooled soil samples representing the four replicates of the Bt and NBt isolines of each corn component, with and without added lignin (+L, −L), from week 1 and week 36. Lignin-derived phenols were liberated from soil using CuO oxidation (Hedges and Ertel, 1982; Otto and Simpson, 2006). Briefly, 0.2–0.4 g of soil was weighed into Teflon-lined bombs with 1 g CuO, 100 mg ammonium iron (II) sulfate hexahydrate, and 15 mL of 2 M NaOH. The bombs were heated at 170 °C for 2.5 h, then cooled immediately under running water. The supernatant was transferred into a teflon centrifuge tube and acidified with 6 M HCl to pH 1, then centrifuged and kept in the dark for 1 h. The supernatant was then transferred into a separation funnel and extracted with diethyl ether, then concentrated and dried under N_2. The CuO oxidation products were derivatized to trimethylsilyl (TMS) derivatives by reaction with 90 mL N,O-bis- (trimethylsilyl)-trifluoroacetamide (BSTFA) and 10 mL pyridine for 3 h at 70 °C before GC/MS analysis. Lignin-derived phenols were measured using an Agilent model 6890N GC with an HP-5MS fused silica capillary column (30 m × 0.25 μm × 0.25 μm thickness) coupled to an Agilent model 5973N quadrupole mass selective detector. The GC operating conditions were as follows: temperature at 65 °C for 2 min, then increased to 300 °C at 6 °C min^{-1}, held at 300 °C for 20 min. The injected sample volume was 3 μl splitless and the run time was 62 min. Agilent Chemstation G1701DA software was used to process the data and identify the compounds by comparison of the mass spectra with the Wiley MS library data. Vanillic acid was used as an external quantification standard and individual compounds were normalized to the amount of organic carbon in each sample. The eight lignin-derived phenols identified were vanillin, acetovanillon, vanillic acid (V units), syringealdehyde, acetosyringone, syringic acid (S units), and *p*-coumaric acid and ferulic acid (C units) (Hedges and Ertel, 1982; Loh et al., 2008; Otto and Simpson, 2006; Poerschmann et al., 2005).

2.4. Stable isotope analysis

Sub-samples of soils from the incubation jars were analyzed for ^{13}C and ^{15}N stable isotopes using a Vario EL III elemental analyzer (Elementar, Germany) with a Conflo II interface (Thermo, Germany) and a Delta XP Plus Advantage isotope ratio mass spectrometer (Thermo, Germany). The international standards Vienna Pee Dee Belemnite (VPDB) ^{13}C with an absolute isotope ratio $^{13}C/^{12}C$ = 0.0112372 and Air with an absolute isotope ratio $^{15}N/^{14}N$ = 0.003676 were used for $\delta^{13}C$ or $\delta^{15}N$ analysis. The proportion of residue-C or residue-N (P, in %) that was recovered in the soil after 36 weeks was calculated as follows:

$$P_{C\ or\ N} = \frac{\delta_{tr} - \delta_C}{\delta_R - \delta_C} \times 100 \quad (3)$$

where δ_{tr} is the $\delta^{13}C$ or $\delta^{15}N$ measured in the treatment soils, δ_C is the $\delta^{13}C$ or $\delta^{15}N$ measured in the control soil which received no

residue ($\delta^{13}C = -24.00$ or $\delta^{15}N = 14.12$), and δ_R is the $\delta^{13}C$ or $\delta^{13}N$ of the corn residue + exogenous lignin where applicable (Table 1) weighted for the proportion of residue/exogenous lignin. The proportion of residue-C and residue-N retained in soil from the added residue (+exogenous lignin where applicable) was calculated as:

$$\text{Proportion residue} - \text{C or } - \text{N retained} = \frac{P_{C \text{ or } N} \times OC \text{ or } N}{C_i \text{ or } N_i} \times 100 \quad (4)$$

where OC is the organic C content of soil (g C kg^{-1} soil), which was assumed equal to total C since no carbonates were detected upon treatment of soil with 1 M HCl, N is the total N content of the soil (in g residue-N kg^{-1} soil), C_i and N_i are the initial amounts of C (g C kg^{-1} tissue) and N (g N kg^{-1} tissue) which was added in the 0.5 g residue and 0.1 g exogenous lignin. Organic C and total N were measured in each treatment replicate at 36 weeks.

2.5. Statistical analysis

All data was checked for normality and log transformed where needed based on the Shapiro–Wilk test. The effects of the plant component (leaves, stems, and roots), corn isolines (Bt, NBt) and lignin addition (+L, −L) on cumulative CO_2 production and N mineralization were tested using the GLM procedure on SAS software (SAS Institute Inc., 2009). Pre-planned orthogonal contrasts were used to evaluate differences between corn components. When effects of corn isolines and lignin addition were significant ($P < 0.05$), mean values were compared with a post-hoc LSMeans test at the 95% confidence level. Decomposition rate constants (k) were calculated by least-squares iteration using the NLIN procedure on SAS software (SAS Institute Inc., 2009). Pearson correlation coefficients (Proc Corr procedure) were used to examine the relation of CO_2 with corn chemical parameters %N, %lignin, C:N ratio, and lignin:N ratio. The Proc Reg procedure (SAS Institute Inc., 2009) was used to relate CO_2 production to the total amount of lignin that was added to each treatment (based on the initial lignin content of the specific plant component and the added exogenous lignin). No statistical analysis was conducted on CuO analyses, performed without replication. Values presented in tables and figures are untransformed means with the standard error of the means (SE).

3. Results

Roots tended to have a greater lignin content, a smaller C content, and a greater lignin:N ratio than leaves and stems (Table 1). The C:N and lignin:N ratios tended to be lower in the Bt tissues than in NBt tissues (Table 1).

Decomposition reached a constant rate after 20 weeks of incubation. There were no significant interactions between lignin and the Bt effect so these results are not presented. Cumulative CO_2 was lower ($P < 0.0001$ at $\alpha = 5\%$) from soils amended with roots than from soils with stems or leaves (Table 2). In leaf- and root-amended soils, there was no difference between Bt and NBt treatments (Table 2), but in stem-amended soil, there was greater CO_2 production from the Bt treatments ($P = 0.044$ at $\alpha = 5\%$) and from the soils without the added lignin ($P = 0.040$). Regression analysis indicated a strong inverse relationship between the amount of lignin in residue and the amount of CO_2 produced in the −L samples ($R^2 = 0.9610$). The slopes of the regression lines (Fig. 1), −1.65 (SE = 0.71) for the +L samples and −1.97 (SE = 0.20) for the −L samples, indicate no difference in the rates of decomposition with the addition of the exogenous lignin. Pearson correlation coefficients showed that CO_2 inversely correlated with lignin:N ratio ($r = -0.933$, $P < 0.0001$, $n = 12$) and indigenous residue lignin ($r = -0.843$, $p = 0.0006$, $n = 12$). CO_2 also correlated well with initial plant N ($r = 0.799$, $P = 0.0018$, $n = 12$) but not with the C:N ratio ($r = -0.456$, $P = 0.14$, $n = 12$).

Total N mineralization (NH_4–N + NO_3–N) after 36 weeks was lowest in root-amended soil and highest in leaf-amended soil (Table 3). Root-amended soils showed the smallest amount of mineralized N followed by stem- and leaf-amended soils (Table 3). The Bt genetic modification negatively affected the amount of N mineralized in root-amended soils (Table 3). The addition of indulin lignin had no effect on the amount of mineralized N in all residue-soil mixtures. Fitting the N mineralization data with a first order equation revealed that the mineralization rate constant k was lowest in root-amended soils ($k = 0.06$–0.07, Table 3). Leaf- and stem-amended soils had comparable k values except for NBt stems that had k values comparable to the root-amended soils (Table 3).

The Ad/Al ratios of vanillin and syringyl increased from 1 to 36 weeks within each treatment suggesting enhanced lignin oxidation over the course of the experiment (Table 4). At 36 weeks, the +L samples had smaller Ad/Al ratios than −L samples in the leaf-amended soils however, this trend was not observed in the stem- and root-amended soils. Comparisons between the Bt and NBt treatments at the beginning of incubation and at the end of the 36 weeks shows that the Bt-amended soils had lower Ad/Al ratios than the NBt-amended soils in all but two treatments within the leaf- and stem-amended soils. This result indicates that the lignin in the NBt residues was initially more susceptible to biodegradation and that it has undergone more modifications to the phenylpropanoid units than the lignin in the Bt residues. The addition of lignin to residue-soil mixtures increased the V:S ratio (Table 4). The V:S ratio increased between week 1 and week 36 of the incubation in leaf- and root-amended soils, but not in stem-amended soils (Table 4).

The amounts of residue-C and residue-N that were retained in the soil after 36 weeks are given in Table 5. There were no

Table 2
Effect of Bt gene modification and addition of alkali lignin to a soil-corn residue mixture on cumulative CO_2 production (g CO_2–C kg^{-1} soil) after 20 weeks. Values are the mean ± standard error ($n = 8$).

Corn residue	Cumulative CO_2 (g CO_2–C kg^{-1} soil)					
	Bt	NBt	Pr > F at $\alpha = 0.05$	+L	−L	Pr > F at $\alpha = 0.05$
Leaves	2.47 ± 0.11	2.39 ± 0.10	NS*	2.41 ± 0.11	2.46 ± 0.10	NS
Stems	2.35 ± 0.06	2.14 ± 0.09	0.044	2.14 ± 0.10	2.36 ± 0.05	0.040
Roots	1.77 ± 0.17	1.79 ± 0.07	NS	1.72 ± 0.10	1.83 ± 0.15	NS
	Contrast analysis (significance probability)					
Leaves vs. Stems	$P = 0.5233$	$P = 0.0557$		$P = 0.0931$	$P = 0.5280$	
Leaves vs. Roots	$P = 0.0007$	$P < 0.0001$		$P = 0.0002$	$P = 0.0006$	
Stems vs. Roots	$P = 0.0032$	$P = 0.0084$		$P = 0.0110$	$P = 0.0029$	

* Not Significant ($P > 0.05$).

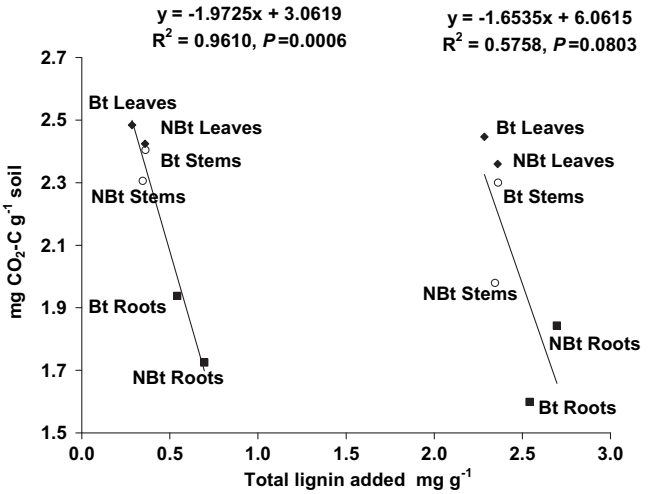

Fig. 1. Cumulative CO_2–C produced after 20 weeks of incubation in relation to total amount of lignin (mg g^{-1}) added to treatments.

differences in the retained residue-C between the +L and −L or the Bt and NBt treatments, however more residue-C was retained from roots ($P = 0.0004$ at $\alpha = 5\%$) than from leaves and stems. The Bt genetic modification and the addition of exogenous lignin significantly affected the proportions of residue-N retained in the soil after 36 weeks of incubation and had a significant interaction between component and genetic modification and between component and exogenous lignin. More residue-N was retained from leaves and stems from NBt plants than Bt plants ($P < 0.0001$ at $\alpha = 5\%$) and more residue-N retained from +L stems than −L stems ($P = 0.0064$ at $\alpha = 5\%$) (Table 5).

4. Discussion

The results support the observation that corn roots have greater lignin contents than stems and leaves, at least at stages before maturity, in agreement with Fang et al. (2007), who reported a lignin content of 11.7% (Bt corn roots) and 9.94% (NBt corn roots). As plants reach maturity and their constitutional lignin deposition is completed, the lignin content of stems is expected to increase to levels close to that of roots. Johnson et al. (2007) and Yanni et al. (unpublished data) found that mature stems from field-grown corn had a similar lignin content as roots, and these components contained more lignin than leaves. It is also possible that stems of field-grown plants may have higher lignin than those from greenhouse plants because more structural rigidity is required in the field. Roots, both Bt and NBt, had lower C content than leaves and stems. This observation agrees with Fang et al. (2007) and Johnson et al. (2007), and can be explained by the fact that only about 25% of the carbon assimilated by plants is transported to the roots (Mooney, 1972). However, it should be noted that there is always a risk of soil contamination in root samples, which could have contributed to the lower C concentration in the roots compared to other plant parts. Corn leaves contained more C and N than stems or roots.

There tended to be smaller C:N and lignin:N ratios in Bt tissue than NBt tissue, due to greater N content in leaves and stems or lower C content in roots. This leads to the question why does Bt tissue tend to have more N than NBt isolines? The presence of the Cry1Ab protein in Bt corn could be the reason. Escher et al. (2000) reported slightly more N content in leaves of X4334-EPR Novartis Bt hybrid (1.2% N) than in a corresponding NBt hybrid (1.1% N) where the Bt hybrids contained 0.46–0.51 mg Cry1Ab g^{-1} dry leaf tissue. The hybrid used in this study was a MON810 Bt, which is reported to contain 7.9–10.3 mg Cry1Ab g^{-1} fresh weight leaf (Canadian Food Inspection Agency, 1997); there was 26% more N in leaves and 48% more N in stems of the Bt hybrid than the NBt isoline, which is consistent with these reports.

Based on lignin content, the roots are expected to have slower decomposition rates than other components and this is evident in the CO_2 and N mineralization data. In this study lignin:N ratio, lignin content, and N content of corn tissue were good predictors of decomposition (cumulative CO_2 production during 20 weeks) and of cumulative N mineralization. Material with a high lignin:N ratio was reported to have slower decomposition rates than material with a low lignin:N ratio (Johnson et al., 2007; Melillo et al., 1982; Taylor et al., 1989), which is consistent with our results. The slower decomposition of roots is also confirmed by the ^{13}C data which shows that more residue-C was retained from roots than other components. It should be noted that loss of ^{13}C from the soil (less residue-C recovery) indicates loss of CO_2, which can be directly linked to the decomposition of organic materials in the jars and a greater amount of ^{13}C recovery in some treatments indicates less degraded plant material. Soil N cycling on the other hand provides several pathways for ^{15}N loss from the soil; when plant material degrades, the released N (and ^{15}N) can be transformed into mineral N forms, incorporated into microbial biomass, or lost from the soil through nitrification (N_2O) and denitrification (NO_x, N_2O, N_2). However, when comparing treatments and when corroborated by CO_2 and N mineralization results, a soil that has more residue-N recovery (less ^{15}N loss) can be said to have less degraded plant material and less gaseous N lost from the system compared to other treatments indicating less decomposition of tissue in the former.

Table 3
Effect of the Bt gene and addition of alkali lignin to a soil-corn residue mixture on cumulative mineral N (mg N kg^{-1} soil) produced after 36 weeks and decomposition rate constant (K) from NLIN estimation. Values are the mean ± standard error ($n = 8$).

Corn residue	Cumulative mineral N (mg N kg^{-1} soil)					
	Bt	NBt	Pr > F at $\alpha = 0.05$	+L	−L	Pr > F at $\alpha = 0.05$
Leaves	153 ± 2.03	150 ± 1.09	NS	151 ± 1.95	151 ± 1.50	NS
Stems	110 ± 1.58	109 ± 1.76	NS	110 ± 2.06	109 ± 1.11	NS
Roots	85.0 ± 0.82	89.8 ± 1.26	0.0064	87.9 ± 0.77	86.7 ± 1.68	NS
	Contrast analysis (significance probability)					
Leaves vs. Stems	$P < 0.0001$	$P < 0.0001$		$P < 0.0001$	$P < 0.0001$	
Leaves vs. Roots	$P < 0.0001$	$P < 0.0001$		$P < 0.0001$	$P < 0.0001$	
Stems vs. Roots	$P<0.0001$	$P < 0.0001$		$P<0.0001$	$P < 0.0001$	
	Weekly decomposition rate constant (k)					
Leaves	0.15 ± 0.00	0.14 ± 0.00		0.16 ± 0.00	0.13 ± 0.00	
Stems	0.16 ± 0.01	0.08 ± 0.01		0.14 ± 0.01	0.10 ± 0.01	
Roots	0.07 ± 0.00	0.06 ± 0.00		0.07 ± 0.01	0.06 ± 0.00	

Table 4
Acid to aldehyde (Ad/Al) ratios and vanillyl to syringyl ratio of root- stem- and leaf-amended soils, with and without added lignin (+L, -L) after 1 week and 36 weeks of laboratory incubation.

Ratio	Bt + L Week 1	Bt + L Week 36	Bt-L Week 1	Bt-L Week 36	NBt + L Week 1	NBt + L Week 36	NBt-L Week 1	NBt-L Week 36
				Leaf-amended soil				
Ad/Al_s	1.49	1.57	1.32	2.03	1.45	2.20	1.71	2.26
Ad/Al_v	1.55	1.34	1.68	2.23	1.15	1.75	2.46	3.65
V:S	1.22	1.59	1.02	1.07	1.28	1.15	1.01	1.09
				Stem-amended soil				
Ad/Al_s	1.05	1.79	1.01	1.31	1.57	1.35	1.08	1.58
Ad/Al_v	0.71	1.14	0.95	1.31	1.44	1.65	1.01	1.39
V:S	1.38	1.21	0.71	0.61	1.79	1.30	0.66	0.69
				Root-amended soil				
Ad/Al_s	0.88	1.40	0.96	1.13	1.18	1.55	1.36	1.42
Ad/Al_v	1.09	1.23	1.58	1.58	1.21	1.31	1.87	2.03
V:S	0.80	1.21	0.69	0.77	1.14	1.25	0.80	0.75

Values are based on lignin phenols yields that were normalized to sample mass. For the determination of the V:S ratio, V is the sum of vanillin + vanillic acid + acetovanillon and S is the sum of syringyl + syringic acid + syringaldehyde. Ad/Al_v is the ratio of vanillic acid to acetovanillon and Ad/Al_s is the ratio of syringic acid to syringaldehyde (Otto and Simpson, 2006).

It was hypothesized that there would be no differences in decomposition rates between Bt and NBt corn residue based on the lack of differences in lignin content, which have been confirmed by the CO_2 evolution results and is in agreement with laboratory incubation results reported by Hopkins and Gregorich (2003) and Fang et al. (2007). The only effect of the Bt genetic modification on CO_2 production was in the stem-amended soils, where it exerted a positive effect on the rate of decomposition and which is reflected in the smaller amount of residue-C retained in Bt stem samples and is also corroborated with the much smaller residue-N in those samples. A closer look at the initial chemical composition of the stem residue shows that this effect can be related to the elevated N content, and consequently smaller C:N ratio of the Bt stems (31 g kg^{-1}) compared to the NBt stems (16 g kg^{-1}) rather than to a difference in lignin content. An effect of the Bt genetic modification on N mineralization was observed in the root-amended soil samples; smaller mineralization from Bt roots cannot be explained by the lignin concentration or the C:N ratio but seems related to the smaller N concentration in Bt roots compared to NBt roots. However, no definite conclusions can be drawn from this result due to the possibility of soil contamination in root samples and the fact that the Cry1Ab protein is not produced in the roots and should not have affected the composition of the root tissue. The numerically greater ^{13}C recovery from NBt stem residues, strongly suggests that up to 21% of stem C from the NBt isolines were transformed into a more stable form of soil C compared to 12% of stem C from the Bt residues. It can be assumed that the apparent differences between treatments are based on the mineralization and loss of the labile material in the residue, rather than to the decomposition of lignin, since it has been strongly related to the lignin:N ratio and N content. On the other hand, the increase in the Ad/Al ratios of the lignin phenols have indicated that lignin has been altered by microbial activity by the end of the nine-month experiment and that the NBt residue was more susceptible to degradation, apparently in disagreement with the CO_2 results indicated above. Ertel and Hedges (1984) and references therein, attribute an elevated Ad/Al ratio to microbial oxidation of the phenylpropanoid units to carboxylic acid without necessarily causing cleavage of the lignin aromatic ring. An increase in carboxyl content could lead to greater solubility and suggests that samples with higher Ad/Al ratios, such as the NBt corn residues, would be more susceptible to degradation once the lignin molecules start to decompose.

A change in the V:S ratio over time is expected when the syringyl units are preferentially degraded over the vanillyl units by the brown-rot and white-rot fungi. Our results indicate that the increase in the V:S ratio between week 1 and week 36 was similar for Bt and NBt treatments providing an added indication that the decomposition of these residues was not different during this time frame.

The addition of exogenous lignin increased the V:S ratio suggesting that this type of lignin had an elevated vanillyl content and is more stable compared to the indigenous corn-derived lignin; therefore it was not expected to decompose or contribute to the production of CO_2 in the +L samples. This was confirmed by the CO_2 production and N mineralization results, which showed no difference between +L and −L treatments except in the +L stem-amended soils where the addition if indulin lignin decreased the production of CO_2 and is supported by the greater residue-N retained from +L stems compared to −L stem treatments, which lost more ^{15}N through nitrification and/or denitrification processes consistent with more decomposition in the −L stem treatments. It is evident from Fig. 1 that this is caused by the NBt +L stems which produced less CO_2 than the other stem treatments; it seems likely that biodegradation of the NBt stems, which had the smallest N content (16 g kg^{-1}), was affected by the addition of the exogenous lignin in those treatments. The observation that indulin lignin

Table 5
Proportion residue-C and residue-N retained in soil, calculated from equations (3) and (4), in a sandy loam soil amended with Bt and NBt tissues after 36 weeks of laboratory incubation and ANOVA treatment effects at $\alpha = 5\%$. Values are the mean ± standard error ($n = 8$).

	Bt	NBt	+L	−L
		Retained Residue-C (%)		
Leaves $_a$	17.7 ± 2.4	16.8 ± 2.1	14.5 ± 1.8	20.0 ± 2.2
Stems $_a$	12.8 ± 2.6	21.1 ± 2.0	17.6 ± 3.0	16.2 ± 2.5
Roots $_b$	31.7 ± 5.8	30.6 ± 5.4	26.5 ± 6.0	35.8 ± 4.6
		Retained Residue-N (%)		
Leaves	66.6 ± 4.3	91.7 ± 2.9*	83.3 ± 4.7	75.0 ± 6.7
Stems	42.8 ± 3.3	100 ± 6.0*	81.1 ± 12.8	62.0 ± 9.6*
Roots	75.3 ± 5.0	71.8 ± 6.2	75.4 ± 6.4	71.7 ± 4.7

Treatment Effects at $\alpha = 5\%$.		
	Retained residue-C	Retained residue-N
Bt	NS	$P < 0.0001$
Lignin	NS	$P = 0.0064$
Component	$P = 0.0004$	NS
Bt × Component	NS	$P < 0.0001$
Lignin × Component	NS	$P = 0.0147$

*Values with different subscripts within a column are statistically different at $\alpha = 5\%$. Values with an asterisk within a row (Bt vs. NBt and +L vs. −L) are statistically different.

apparently did not decompose during the incubation period is not surprising since it is a recalcitrant material and in a different chemical form than lignin in corn tissue. Indigenous lignin is bound to other fibers and proteins and forms complexes with hemicellulose and cellulose; plant lignin undergoes biochemical modifications and is released gradually as cells are broken down by decomposers, which is not the case for indulin lignin. This is probably why the total amount of lignin (+L samples) showed a non-significant regression relationship with CO_2 production whereas indigenous lignin (−L samples) significantly related to CO_2 production.

We conclude that lignin and N contents, and consequently the lignin:N ratio, of corn tissue control its decomposition. The C:N ratio of stem residue appears to explain the positive effect of the Bt genetic modification on decomposition and N mineralization from these plant components. Corn roots, which accumulate a biomass of 3000–5000 kg ha^{-1} (Prince et al., 2001) and have an average of 62 g kg^{-1} lignin, could make an important contribution to soil C because of their inherent resistance to decay. Aboveground corn residues (stems and leaves) were more susceptible to decomposition in this study. This might not be the case in corn agroecosystems due to the similarity in lignin content of corn roots and stems at physiological maturity. In addition, the effect of herbivory by European corn borer on the physical strength and integrity of NBt corn residues needs to be considered under field conditions. Since exogenous lignin was not susceptible to decomposition and generally did not contribute to more CO_2 production and N mineralization, we were only able to observe the effect of elevated-lignin residue on decomposition through the root decomposition patterns, which exhibited about 50% slower decomposition rates. The results strongly suggest that Bt corn does not differ from NBt corn in terms of decomposition and should have no effect on the soil C dynamics in Bt corn agroecosystems.

Acknowledgements

Financial support was from the Green Crop Network, funded by the Natural Sciences and Engineering Research Council of Canada.

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