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REGULAR ARTICLE

Seven years of continuously planted Bt corn did not affect mineralizable and total soil C and total N in surface soil

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Abstract Genetically engineered corn (*Zea mays* L.) containing a gene from the soil bacterium Bt (*Bacillus thuringiensis*) constitutes a large proportion of all corn planted in the United States. In a number of studies, Bt plant residues have been reported to have higher lignin content and to decompose slower than those of non-Bt plants, possibly due to the presence of the Cry endotoxin. We hypothesize that after multiple years of continuous cultivation of Bt corn, the combined results of alleged differences in Bt corn residues and of Cry endotoxin presence will be reflected in soil characteristics, specifically in an increase in soil total carbon (C) and nitrogen (N) levels. We collected soil samples at 0–7.5 cm depth in 2006 from continuous Bt and non-Bt corn treatments in an randomized complete block design experiment with four replications. The experiment was established in 1999 at the Kellogg Biological Station long-term ecological research (LTER) site in southwest Michigan. We found that, after 7 years of Bt cropping, neither total soil C and N nor soil C mineralized during a 35-day incubation were significantly different between Bt and non-Bt corn treatments ($P>0.05$). Total soil C was equal to 7.3 g kg^{-1} and 7.4 g kg^{-1}, in Bt and non-Bt corn, respectively, with a standard error of the means (SEM) = 0.2, and total N was 0.67 g kg^{-1} (SEM 0.02) in both treatments. Post-hoc power analysis indicated that, given the number of samples collected in this study and the observed level of variability, the minimal differences between the Bt and non-Bt treatments that could be detected as statistically significant at $\alpha=0.05$ with a power of 0.80 were equal to 1.0 g kg^{-1}, 0.14 g kg^{-1} and 0.125 g kg^{-1} for total C, total N, and soil C mineralized during a 35-day incubation, respectively. The results indicate that continuous Bt corn production during a 7-year period did not lead to sizeable changes in total soil C and N.

Keywords *Bacillus thuringiensis* · Bt corn · Mineralizable C · Total soil C · Total soil N

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Introduction

The area planted to genetically engineered corn containing a gene from the soil bacterium Bt (*Bacillus thuringiensis*) has been increasing continuously since 1996 when it was first introduced; in 2008, Bt corn made up 57% of all corn planted in the United States (USDA Economic Research Service 2008). The effects of Bt corn on soil biota have been addressed in a number of studies and most reports show that Bt corn is less damaging to soil biota than traditional insecticide-based management (e.g., Marvier et al. 2007). Differences in soil microbial communities under Bt and non-Bt corn have also been observed (e.g., Donegan et al. 1995; Blackwood and Buyer 2004; Castaldini et al. 2005; Griffiths et al. 2005), and have been related in part to the presence of the Cry endotoxin in Bt corn residues as well as to transfer of the endotoxin to soil as plant root exudates (Saxena et al. 2002). Differences in plant residue composition have been reported; Bt plant residues tend to have a higher lignin content than non-Bt varieties (Saxena and Stotzky 2001; Poerschmann et al. 2005; Flores et al. 2005; Fang et al, 2007). However, lack of differences between Bt and non-Bt in terms of microbial community structure and lignin content have also been reported (e.g., Jung and Sheaffer 2004; Mungai et al. 2005; Shen et al. 2006; Lehman et al. 2008; Icoz et al. 2008; see Icoz and Stotzky 2008 for an up-to-date review). Reported studies of the decomposition of Bt residues in soil give inconsistent results. Some studies report no difference in decomposition rates (Hopkins and Gregorich 2003; Cortet et al. 2006; Zwahlen et al. 2007; Lehman et al. 2008), others have measured slower decomposition of Bt residues in all (Castaldini et al. 2005; Flores et al. 2005) or some (Mungai et al. 2005) of the studied residue types.

One of many possible sources of inconsistency in the results may be the longevity of the studied agricultural systems. To date, field studies have considered soils from experiments where Bt corn has been grown for fewer than 2–4 years. However, slower decomposition of the Bt residues and its effect on soil properties may not become apparent in such short-term laboratory or in situ field experiments. We hypothesize that, even if effects are not detectable in the short term, over longer periods of time the alleged differences in residue properties, differences in microbial communities, and presence of Cry endotoxin may manifest themselves through differences in soil properties, and in particular in soil organic matter content. Specifically, soil under long-term continuous cultivation of Bt corn is hypothesized to have higher total C and N than soil under continuous non-Bt corn. The objective of the present study was to assess whether longer-term (7 years) continuous Bt corn planting can lead to increases in mineralizable C and in total soil C and N in surface soil as compared to conventionally managed non-Bt corn. The soil sampling depth for the analysis was selected as 0–7.5 cm. The top soil layer is where the differences between Bt and non-Bt corn are most likely to first appear, since this layer includes not only residues incorporated by plowing but also fresh residues and denser plant roots.

Materials and methods

Our study was conducted at the Kellogg Biological Station (KBS) long-term ecological research (LTER) site in southwest Michigan (42° 24′ N, 85° 24′ W). Soils developed on glacial outwash and are classified as well-drained, Typic Hapludalfs coarse-loamy, mixed, mesic (Oshtemo series). The clay mineralogy of KBS soils is dominated by chlorite and illite (Six et al. 2000). Soil characteristics of the site are pH of 7.1, available P by Bray and Kurtz P-1 method of 80 kg ha^{-1}, and exchangeable K and Ca by procedures described in North Central Regional Research Publication No. 221 (1998) of 200 and 1,867 kg ha^{-1}, respectively (http://houghton.kbs.msu.edu/data/agronomic_protocol/2007AgronomicProtocol.pdf; KBS 2007).

The long-term cropping systems experiment was established in 1999. A full description of the experiment with agronomic protocols is given on the LTER website (KBS 2007). The experimental design was a randomized complete block with four replications. Plots are 9×23 m. The two studied treatments are continuous Bt corn and continuous non-Bt corn with insecticide pest control for European corn borer. Before 2003, the varieties Bt Pioneer 36G13 and non-Bt Pioneer 36G12 were used; after 2003 these were replaced with Bt Pioneer 36B09 and non-Bt 36B08. These Bt varieties express Cry1Ab protein, which is active against European corn borer (event MON810). The plots are tilled by conventional chisel ploughing. Fertilizer and insecticide applications were made according to Michigan State University soil test and pest control recommendations.

In August 2006, four undisturbed soil samples (7.5 cm in diameter×7.5 cm deep) were collected from each plot at 0–7.5 cm depth using a double-cylinder bulk density core sampler. Within 4 h of collection the samples were transported to the laboratory and kept at 4°C until analysed. From each

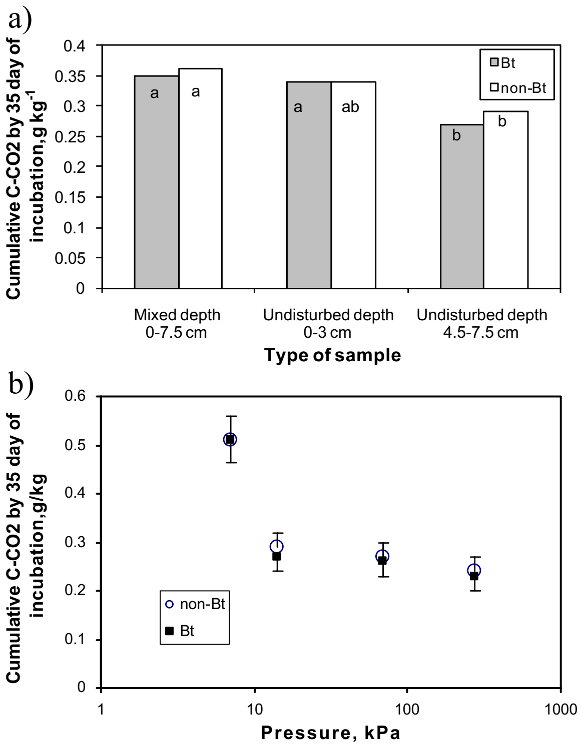

Fig. 1 Means of the cumulative CO_2-C respired from soil samples from a continuous 7-year Bt and non-Bt corn experiment at the end of a 35-day incubation across **a** all moisture potentials, and **b** all sample types. In **a** means of different sample types within the same treatment (Bt or non-Bt) with the same lower case letters are not significantly different ($P<0.05$); in all sample types there was no significant difference between Bt and non-Bt. In **b** *error bars* represent standard errors; for clarity the *top bar* is for non-Bt and the *bottom bar* is for Bt

sample, we cut two undisturbed sub-samples, one undisturbed sample from 0–3 cm depth and another undisturbed sample from 4.5–7.5 cm depth. The undisturbed samples were in brass rings 5.5 cm in diameter×3 cm height. The additional disturbed sample was composited from the loose mixed soil remaining after cutting out the undisturbed samples and represented the entire 0–7.5 cm depth. The soil for the disturbed sample was passed through a 2 mm sieve and packed into the brass ring to a weight equal to the average of the weights of the two undisturbed samples in order to maintain the same bulk density as that in the undisturbed soil.

The samples were saturated from the bottom and then brought to a pressure (suction) of either 7, 14, 69, or 276 kPa (Klute 1986). After reaching equilibrium, the samples were subjected to incubation at a constant temperature of 25°C, following the method described by Robertson et al. (1999). The CO_2 respiration rate was first measured 3 days after the beginning of the incubation and then every 7 days using an infra-red gas analyzer (LICOR-6252; LICOR, Lincoln, NE) for a total of 35 days. The overall amount of CO_2-C respired by the end of the incubation was regarded as mineralizable C. The remaining loose material from each sample was used for measurements of soil texture using the hydrometer method (Gee and Bauder 1986); total C and N were measured using a Carlo-Erba C-N analyzer (Carlo Erba Instruments, Milan, Italy).

Data analyses were conducted using the PROC MIXED module of SAS (SAS 2002). For the incubation data the statistical model included fixed effects of treatment (Bt and non-Bt corn), pressure, sample type (undisturbed 0–3 cm, undisturbed 4.5–7.5 cm, and mixed), and the interactions between them. The random effects consisted of effect of blocks, plots, undisturbed 7.5 cm×7.5 cm samples, and 5.5 cm×3 cm sub-samples, the latter three were used as error terms for testing the effects of treatment, pressure, and sample type, respectively. For total C and N, the model included treatments as a fixed effect and blocks, plots, and undisturbed 7.5 cm×7.5 cm samples as random effects, with plots being used as an error term for testing the treatment effect. Normality and homogeneous variance assumptions were checked and, when found unsatisfactory, the data were either log-transformed or analyses with heterogeneous variances were conducted. The effects and differences were declared as statistically significant at $P<0.05$.

Post-hoc power analysis was conducted using the approach outlined by Stroup (2002). Calculations were based on the numbers of replications and sub-samples used in the study and the obtained estimates of the variance components. We computed the minimal differences between Bt and non-Bt corn treatments that could be declared as statistically significant at $\alpha=0.05$ with power of 0.80.

Results

The results indicated that there was no significant difference in soil CO_2-C respired between Bt and non-Bt treatments at either of the incubating suctions

Table 1 Averages and standard errors (in parentheses) for sand, silt, and clay contents of the studied soil along with total N and total C after 7 years of continuous cultivation of Bt and non-Bt corn. For all properties Bt and non-Bt corn treatments were not significantly different from each other ($P<0.05$). Soil is Typic Hapludalfs coarse-loamy, mixed, mesic (Oshtemo series)

Treatment	Sand, g/kg	Silt, g/kg	Clay, g/kg	Total N, g/kg	Total C, g/kg
Bt	575 (27)	341 (27)	85 (4)	0.67 (0.024)	7.3 (0.21)
Non-Bt	597 (27)	328 (27)	75 (4)	0.67 (0.023)	7.4 (0.20)

or in any of the sample types (Fig. 1). The surface soil sample (0–3 cm) and the mixed soil sample (0–7.5 cm) had very similar levels of CO_2-C respired, significantly higher than those observed in the undisturbed subsurface sample (4.5–7.5 cm) (Fig. 1a).

As expected, the highest CO_2 respiration was observed at a suction of 7 kPa, which provided the most optimal moisture conditions for soil microbial activity (Fig. 1b). However, further increase in suction did not lead to a further decrease in CO_2 respiration.

Neither total C nor total N were significantly affected by continuous Bt corn growth during the past 7 years (Table 1) ($P>0.05$). The differences in mean values were very small, e.g. ,total soil C was equal to 7.3 g kg^{-1} in Bt treatment and to 7.4 g kg^{-1} in non-Bt corn, while total N was 0.67 g kg^{-1} in both treatments.

Discussion

The decrease in soil respiration with depth that we observed reflects the presence of plant residues on the soil surface. Similar results have been reported in a number of other studies (e.g., Paul et al. 1999; Collins et al. 2000).

A result that lacks statistical significance can originate both from insufficient sample size as well as from absence of difference. To identify what is the size of the differences in total and mineralizable C and in total N that could be detected in this study we conducted a power analysis. This analysis showed that, given the numbers of replications and sub-samples of this study and the observed variability (Table 2), the minimal difference in the cumulative CO_2-C respired during 35 days between the two treatments that could be detected as statistically significant at $\alpha=0.05$ with power of 0.80 is equal to 0.125 g kg^{-1} soil. Based on the power analysis, and considering this study's observed variability (Table 2), the minimal difference in total C between the two treatments that could be detected as statistically significant at $\alpha=0.05$ with power of 0.80 is equal to 1.0 g kg^{-1} soil. The minimal difference that could be detected for total N is 0.14 g kg^{-1}. These minimal detectable differences are smaller than the differences observed in this soil in previous studies that examined different management practice effects on soil C at the KBS LTER site. For example, Hao and Kravchenko (2007) reported that, after 15–16 years of two conservational management practices, i.e., no-till management and organic management with legume cover crops, total C and N were 3–4 g kg^{-1} higher and 0.2–0.4 g kg^{-1} higher, respectively, than those of the conventional chisel plow management practice. This indicates that total C and N content of the studied soil can be increased when soil disturbance is reduced or when cover crops are used. However, continuous Bt corn production during a somewhat shorter but comparable time span did not lead to changes in soil C and N.

The lack of differences observed in this study is consistent with other studies that reported minor or no significant differences between Bt and non-Bt treat-

Table 2 Estimates of the variance components for CO_2-respired C, and total soil C and N

Variance for:	Cumulative C-CO_2 in 35 days of incubation	Total C	Total N
Blocks	0.0001	0.055	0.0001
Plots	0.0001	0.060	0.0019
Samples	0.0047	0.159	0.0009
Laboratory measurement replications	0.0043	0.077	0.0006

ments in terms of different parameters related to soil organic matter processes, such as lignin content of plant residues, rates of plant residue decomposition, and microbial community structures (Hopkins and Gregorich 2003; Jung and Sheaffer 2004; Mungai et al. 2005; Cortet et al. 2006; Lehman et al. 2008; Icoz et al. 2008). Our results are also consistent with soil data analyses from shorter-term Bt corn experiments (Mungai et al. 2005; Coleman et al. 2006). After a 2-year Bt corn experiment, Mungai et al. (2005) found no differences between Bt and non-Bt corn in terms of either inorganic soil N in the field or N mineralization rates in the laboratory. Coleman et al. (2006) found no differences in soil microbial biomass C 1–2 years after the beginning of a Bt cotton experiment. It is interesting to note that in 2 years of a 3-year field experiment, Devare et al. (2007) found a higher microbial biomass and higher soil respiration rates in Bt corn as compared with non-Bt corn.

Lack of differences could be related to the coarse soil texture of the studied location. Analyses of the relationships between soil C and N with soil sand content conducted by Hao and Kravchenko (2007) at the KBS LTER site revealed that, after 15–16 years of the experiment, larger differences in total soil C and N between conventional and conservational management practices were observed in locations with lower sand content. The differences were smaller when sand content exceeded 430 g kg^{-1}. High sand content at the Bt corn experimental site (550 g kg^{-1}) might have contributed to slower accumulation of differences in soil C and N compared to what could have been achieved at finer textured locations. Having only a single long-term experimental site is a limitation of the present study. Multiple sites located in soils with different parent material and textural compositions would have enabled generalization of the results obtained. However, analysis of literature data did not reveal any consistent patterns in terms of soil texture effects on differences between Bt and non-Bt cropped soils. Results ranging from no and minor differences to significant differences were reported in fine- and coarse-textured soils alike. Unfortunately, published studies vary not only in terms of soil texture but in terms of such crucial variables as experiment duration, crop varieties, soil types, management practices, etc. Overall, our results support the notion, based on a comprehensive analysis of the recent literature, that Bt crops have only minor, if any, effect on soil characteristics (Icoz and Stotzky 2008).

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