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Decomposition processes under *Bt* (*Bacillus thuringiensis*) maize: Results of a multi-site experiment

Jérôme Cortet, Mathias N. Andersen, Sandra Caul, Bryan Griffiths, Richard Joffre, Bernard Lacroix, Christophe Sausse, Jacqueline Thompson, Paul Henning Krogh

correspondance: jerome.cortet@univ-montp3.fr

Abstract

The effects of maize expressing the *Bacillus thuringiensis* Cry1Ab protein (*Bt* maize) on decomposition processes under three different European climatic conditions were assessed in the field. Farming practices using *Bt* maize were compared with conventional farming practices using near-isogenic non-*Bt* maize lines under realistic agricultural practices. The litter-bag method was used to study litter decomposition and nitrogen mineralization dynamics of wheat straw. After 4 months incubation in the field, decomposition and mineralization were mainly influenced by climatic conditions with no negative effect of the *Bt* toxin on decomposition processes.

Keywords: Wheat straw decomposition; Nitrogen mineralization; Maize; Genetically modified plants; *Bacillus thuringiensis* toxin

Maize (*Zea mays* L.) has been genetically modified to express the Cry1Ab protein from the bacterium *Bacillus thuringiensis* to produce a protein that is toxic to some lepidopteran insect pests, particularly the European corn borer *Ostrinia nubilalis*. As the toxin, released to soil from *Bt* maize in root exudates, has been shown to degrade slowly and to accumulate in soil (Tapp and Stotzky, 1995, 1998; Saxena et al., 2002; Zwhalen et al., 2003a) it is desirable to assess the effects of *Bt* maize cultivation on non-target soil organisms, similar to the assessment of any other kind of pesticide. Under laboratory conditions, no effect of the Cry1Ab protein was found on Collembola (Sims and Martin, 1997), isopods (Escher et al., 2000), protozoa, nematodes, fungi, bacteria, algae, and earthworms

(Saxena and Stotzky, 2001; Koskella and Stotzky, 2002). However, a small significant effect of this toxin was noticed on soil microbial community structure (using community-level physiological profiles) in a high-clay soil (Blackwood and Buyer, 2004). Studies of microbial and microfaunal communities under two years of *Bt* maize revealed differences between the *Bt* and non-*Bt* crops, but non that were outside the expected variation of agricultural practices (Griffiths et al., 2005). A combination of laboratory, glasshouse and field studies (the so-called 'tiered' approach, Jepson et al., 1994) is required as the toxin can have a different behavior in the field than in the laboratory, and insecticidal activity can vary with climatic, soil texture and pH conditions (Tapp and Stotzky, 1995; Zwhalen et al., 2003b).

Other studies have examined the effects of *Bt* crops on ecosystem functions, such as decomposition. Such a functional effect could have a direct impact on soil fertility, which is important for agriculture (Eijsackers and Zehnder, 1990). Most of these studies have compared

the decomposability of *Bt* and non-*Bt* plant residues. Hopkins and Gregorich (2003) did not observe any detectable difference in the decomposition of plant material from *Bt* and non-*Bt* maize lines, as determined by CO₂ production. Flores et al. (2005) noticed a higher CO₂ soil production with near-isogenic non-*Bt* maize residues than with *Bt* maize residues that could not be explained by differences in C/N ratio, lignin maize content, or even soil microbiota. This approach is useful for determining whether there is any inherent difference in the decomposability of plant material containing the *Bt* toxin, but could not assess whether the presence of *Bt* crops would influence the decomposition of exogenous organic matter. We addressed this using a litter-bag technique. Litter-bags containing wheat straw have been suggested as a suitable field test method for assessing the effects of pesticides on decomposition processes (Kula and Römbke, 1998; Cortet et al., 2002; Knacker et al., 2003) and were recently presented as a standard test for soil functioning employed in arable fields (Römbke et al., 2002). We focus here on the effects of *Bt* maize on decomposition processes by using this litter-bag methodology under realistic agricultural conditions. The main objective was to assess the overall crop effects (i.e., *Bt* or non-*Bt* maize) using wheat straw (*Triticum aestivum* L.) as a standard exogenous material in the litter-bags.

Field locations, selected to represent diverse climatic and soil conditions, were part of the ECOGEN project (www.ecogen.dk) and also used for the analysis of soil communities (Griffiths et al., 2005). Foulum (Jutland, Denmark) was the most northerly site at 56°30'N, 9°35'E. Varois (Bourgogne, France) was an intermediate geographic site at 47°34'N, 5°13'E. Narbons (Midi-Pyrénées, France) was the most southerly site at 43°26'N, 1°27'E. Soil properties are summarised in Table 1. At Foulum and Varois two maize cultivars were planted: MEB307 (a *Bt* variety producing the Cry1Ab toxin) and Monumental (the conventional near-isogenic variety without the *Bt* trait) each randomised in plots of at least 12 m × 19 m in a four-block design. Fields were ploughed in the autumn of 2002, seeded on 22 and 28 May 2003, and harvested on 5 November and 18 October, in Foulum and Varois, respectively. All fertilization and pesticide applications were done according to current agricultural practices and were identical for the two treatments. The same protocols (with *Bt* and near-isogenic varieties) had been previously applied in 2002 on the same plots in Foulum and Varois. A similar design was used in Narbons but the maize cultivars, suited for the regional

climate, were DK552Bt (a *Bt* variety expressing the Cry1Ab toxin) and DK532 (the conventional near-isogenic variety without the *Bt* trait). The field was ploughed and seeded on 4 June 2003 and harvested on 10 October 2003. In Narbons, the seeds of the near-isogenic maize variety were treated with Gaucho (0.112 kg/ha, active ingredient imidaclopride), whereas the *Bt* maize seeds received no insecticide. All field operations were identical for both treatments. Plots were irrigated regularly throughout the growing season at Narbons but not at Foulum and Varois. Wheat straw decomposition was assessed using litter-bag methodology (Cortet et al., 2002). The same sample of straw, hand-picked to select only internodes, was used for all three sites. It was cut into small pieces (5–10-cm pieces), oven-dried (45 °C for 48 h) and 5 g portions sealed in 12 cm × 12 cm Nylon bags (5 mm diameter mesh). Chemical analysis of five replicates of initial straw gave coefficient of variation (%) of 0.6, 2.8 and 2.9 for C, N and ash, respectively. Bags were installed in the field just after maize emergence and collected at regular intervals until harvest. Twenty-four bags were placed on top of the soil in the centre of each plot, within rows but between the plants. In Foulum, eight bags per plot were destructively sampled 51, 101, and 142 days after exposure. In Varois and Narbons four bags per plot were destructively sampled 43, 93, and 126 days and 43, 85, and 121 days after exposure, respectively. Wheat straw from the bags was oven-dried (24 h, 45 °C), sieved (0.5 mm) to eliminate most soil particles, weighed and ground (1 mm mesh filter) with a Cyclotec-1093 mill (Tecator, Höganäs, Sweden). All samples (total of 384) were analyzed using a near-infrared spectrophotometer (NIRS system 6500, Foss Nirs systems, Inc., Silver Spring, MD, USA). Ash-free litter mass remaining (LMR), N concentrations and C/N were predicted from calibrations equations derived from ash and wet chemistry measurements done on 52 representative samples (see Joffre et al., 1992; Coûteaux et al., 1998; Cortet et al., 2003 for methodological details). As litter-bags collected at a given date ($t+n$) cannot be considered as independent from litter-bags collected at the previous date (t), samples were considered as repeated measures (Grafen and Hails, 2002). LMR, N concentrations, and C/N were each compared using a global two-way MANOVA (Analysis of variance with multiple variables), with sites (Foulum, Varois and Narbons) and crops (*Bt*/non-*Bt*) being fixed effect factors, and dates being the multiple variable (three sampling dates). We considered the intervals between the three sampling dates to be equivalent for each site. For each site, LMR, N concentration and C/N were also each compared using a two way MANOVA, with crops (*Bt*/non-*Bt*) being fixed effects factors, blocks being a random factor, and dates being the multiple variable (three sampling dates) (Statgraphics plus 5.0 software, Manugistics Corp., Rockville, MD, USA).

The time-course of LMR, N concentrations, and C/N showed similar trends among the three sites (Fig. 1). There was a significant site effect (Table 2) with a greater

Table 1
Soil properties of the three sites, Foulum, Varois and Narbons

	Clay (%)	Silt (%)	Sand (%)	Organic matter (%)	Calcium (%)	pH (H ₂ O)
Foulum	8.7	24.8	66.5	6.4	0.0	6.2
Varois	43.1	39.0	17.9	4.8	18.8	8.1
Narbons	27.9	38.3	33.8	1.5	7.0	8.2

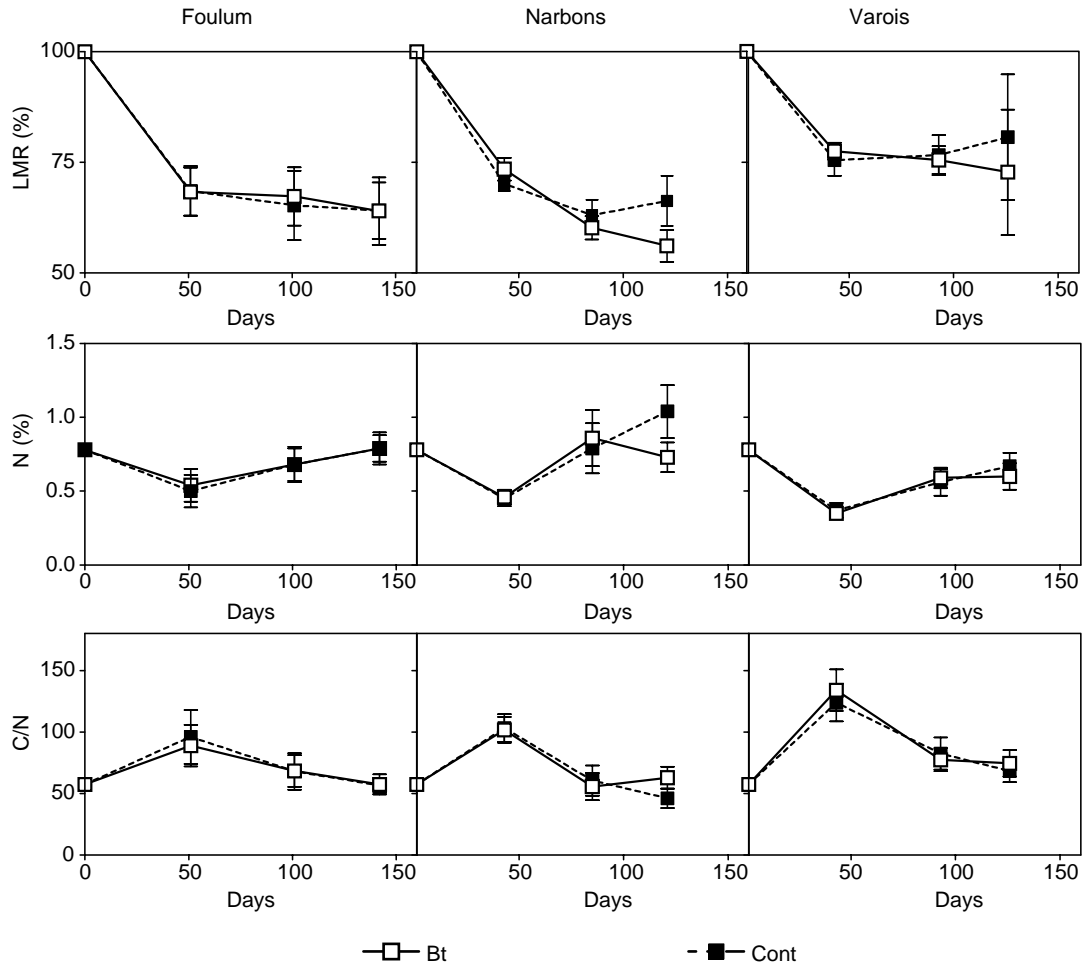


Fig. 1. Litter mass remaining, total N concentration and C/N dynamics of the wheat straw (*Triticum aestivum* L.) residues at the three European sites (Foulum, Varois and Narbons) under *Bt* (open symbol, solid line) and near-isogenic non-*Bt* (open symbol, dashed line) maize crops. Vertical bars indicate the standard deviation of the mean.

decomposition (opposite to LMR) at Narbons compared with Foulum and Varois, and at Foulum compared with Varois. Across all three sites, at the end of the study, decomposition was shown to be greater under *Bt* than non-*Bt* maize (Table 2), although individually, the only significant effect was at Narbons (Table 3). Furthermore, the significant *Bt* effect observed in Narbons was significantly influenced by the block (Table 3). N concentrations showed the opposite trend to decomposition (non-*Bt* > *Bt*), while C/N showed the same trend as decomposition (Fig. 1).

Our results for the three sites agreed with the common finding that straw decomposition depends strongly on climatic conditions and soil properties (Heal et al., 1997). Decomposition was faster in the warmest, irrigated Narbons site compared with the other sites. Our values of LMR agree with previous studies (e.g. Holland and Coleman, 1987; Robinson et al., 1994), indicating a slow decomposition of wheat straw due to a high initial C/N (60 in this case) that inhibits microbial breakdown (Römbke et al., 2002). Crop effect differed significantly between sites for the three

studied parameters, indicating that no general conclusions could be drawn over the effect of *Bt* maize on decomposition processes (Table 2). Single site MANOVA revealed contrasting effects (Table 3). At Narbons, significant differences were observed between straw decomposed under *Bt* maize compared with the near-isogenic non-*Bt* maize, with an increase in decomposition and N mineralization under DK552*Bt* compared with the near-isogenic DK532 cultivar at the end of the study. A possible explanation includes a variety effect, as a *Bt* containing variety could produce qualitatively different maize compared to its near-isogenic line and, thus, create a different environment for decomposers (Escher et al., 2000). This assumption was also supported by the absence of significant difference in Varois and Foulum where the other varieties, MEB307*Bt* and Monumental, were used. However, this result remains unclear particularly because no difference was observed concerning the microbial community structure or biomass on the same plots in Narbons (Griffiths et al., 2005). However, whether this is a variety effect or not, it can

Table 2

Comparison of LMR (Litter mass remaining), [N] (Nitrogen concentration) and C/N of wheat straw residues under *Bt* maize (Cry1Ab) and corresponding near-isogenic non-*Bt* maize lines and between the sites (two-way MANOVA on 384 samples, crops and sites being the varying factors for each sampling date)

	MANOVA			Multiple comparison (Fisher's lsd ^a)		
	Wilk's lambda	F	P	Date 1 ^b	Date 2 ^c	Date 3 ^d
LMR						
Crop ^e	0.87	5.6	0.0013	<i>Bt</i> > iso	ns ^f	Iso > <i>Bt</i>
Site ^g	0.33	27.4	<0.001	V > N > F	V > F > N	V > N, V > F
Crop × site	0.88	2.4	0.02	<i>Bt</i> N > isoN ^h	ns	IsoN > <i>Bt</i> N
[N]						
Crop	0.76	12.0	<0.001	ns	ns	Iso > <i>Bt</i>
Site	0.31	36.0	<0.001	F > N > V	N > F > V	N > F > V
Crop × site	0.73	6.4	<0.001	ns	ns	IsoN > <i>Bt</i> N
C/N						
Crop	0.81	8.7	<0.001	ns	ns	<i>Bt</i> > iso
Site	0.32	29.1	<0.001	V > N > F	V > F > N	V > F > N
Crop × site	0.82	3.9	<0.001	ns	ns	<i>Bt</i> N > isoN

Analysis of variance with multiple variables (see text for more explanation).

^a lsd, least significant difference.

^b Forty-three decomposition days in Varois and Narbons, 51 days in Foulum.

^c 85, 93 and 101 decomposition days, respectively, in Narbons, Varois, and Foulum.

^d 121, 126 and 142 decomposition days, respectively, in Narbons, Varois, and Foulum.

^e Two crops: *Bt* maize (*Bt*) versus near-isogenic non-*Bt* maize (iso) (MEB 307 and Monumental in Foulum and Varois, DK532*Bt* and DK532 in Narbons).

^f ns, non-significant difference.

^g Three sites: Foulum (F), Varois (V) and Narbons (N).

^h *Bt*N and isoN, respectively, *Bt* maize in Narbons and near-isogenic non-*Bt* maize in Narbons.

Table 3

Comparison of LMR (Litter mass remaining), [N] (Nitrogen concentration), and C/N of wheat straw residues under *Bt* maize (Cry1Ab) and corresponding near-isogenic *Bt* maize lines in three different sites (two-way MANOVA on 384 samples, crops and blocks being the varying factors for each sampling date)

	MANOVA			Multiple comparison (Fisher's lsd ^a)		
	Wilk's Lambda	F	p	Date 1 ^a	Date 2 ^a	Date 3 ^a
Foulum						
LMR						
Crop ^a	0.99	0.01	0.99	ns	ns	ns
Block ^b	0.40	0.13	0.99	ns	ns	ns
Crop × block ^c	0.71	2.1	0.03	ns ^a	ns	Iso2 > <i>Bt</i> 2, <i>Bt</i> 3 > iso3
[N]	No significant differences					
C/N	No significant differences					
Varois						
LMR	No significant differences					
[N]	No significant differences					
C/N	No significant differences					
Narbons						
LMR						
Crop	0.09	3.52	0.36	ns	ns	Iso > <i>Bt</i>
Block	0.38	0.14	0.99	ns	ns	ns
Crop × block	0.17	5.1	<0.001	<i>Bt</i> 2 > iso2	ns	ns
[N]						
Crop	0.25	1.01	0.59	ns	ns	Iso > <i>Bt</i>
Block	0.56	0.08	0.99	ns	ns	ns
Crop × block	0.20	4.2	<0.001	<i>Bt</i> 4 > iso4	<i>Bt</i> 3 > iso3	Iso3 > <i>Bt</i> 3, iso4 > <i>Bt</i> 4
C/N						
Crop	0.26	0.96	0.61	ns	ns	ns
Block	0.57	0.07	0.99	ns	ns	ns
Crop × block	0.23	3.9	0.001	Iso4 > <i>Bt</i> 4	ns	<i>Bt</i> 3 > iso3, <i>Bt</i> 4 > iso4

^a See Table 1.

^b Four blocks numbered from 1 to 4.

^c Iso2, iso3, iso4 are, respectively, near-isogenic non-*Bt* maize for the Blocks 2 to 4. *Bt*2, *Bt*3, *Bt*4 are, respectively, *Bt* maize for the Blocks 2 to 4.

be concluded that under the cropping systems studied *Bt* maize had no negative effect on the decomposition of exogenous organic matter.

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