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Impact of multi-resistant transgenic Bt maize on straw decomposition and the involved microbial communities

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ABSTRACT

A general concern associated with the use of transgenic Bt maize is its possible negative influence on non-target organisms and ecosystem functions such as organic matter transformation. Our study was carried out to assess the impact of the multi-resistant Bt maize hybrid MON 89034 × MON 88017 on straw decomposition and the residue-colonizing microbial communities in comparison to the near-isogenic control and two conventionally bred varieties. Straw decomposition was analyzed by a substrate-induced respiration method and monitoring of the complete decomposition after ^{14}C pulse labelling of the maize plants. Both approaches indicated significant differences in the decomposition rate between the conventional varieties, but the transgenic Bt hybrid and its near-isogenic control could not be distinguished. Potential effects on the residue-colonizing bacterial and fungal communities were analyzed by the quantification of metabolically active microbial groups using taxon-specific primers and T-RFLP analyses of the small-subunit ribosomal RNA gene. Results obtained over three years and three sampling dates did not reveal significant differences between the transgenic hybrid and the control. The abundance of the metabolically active microbial groups varied between the varieties only in some cases and temporarily restricted. Similarly, T-RFLP analysis did not show an impact of the plant genotype on the bacterial and fungal community structure. In contrast, seasonal effects given by different sampling dates as well as varying soil properties within the field trial were identified as drivers of the microbial communities colonizing the rotting maize straw. Conclusively, the multi-resistant Bt hybrid MON 89034 × MON 88017 did not indicate an adverse impact on straw decomposition and the involved microbial communities.

1. Introduction

The cultivation of Bt maize resistant to economically important pests has been steadily increasing since its introduction in 1996. In 2012 transgenic maize varieties, most of which represented Bt maize hybrids, were grown on 55 Mio ha, that is about 35% of the worldwide maize cultivation area (James, 2012). Maize varieties expressing *cry* genes for the production of crystal delta endotoxins of *Bacillus thuringiensis* to control the European corn borer (*Ostrinia nubilalis*) or the corn rootworm (*Diabrotica virgifera*) provide stable yields and a reduced need for application of insecticides (Klaphengst et al., 2011) as well as an improved food safety by lower mycotoxin levels (Folcher et al., 2010; Ostry et al., 2010). To extend protection, various Bt hybrids have been developed that harbour two or more *cry* genes originally present in different parental varieties. These multi-resistant hybrids, also referred to as "stacked events", combine several potentially interacting transgenic traits, and thus represent new transgenic plants which need to be evaluated in light of ecological risk assessment. A general concern associated with the cultivation of Bt maize is its possible adverse impact on the agro-ecosystem. Both the production of the insecticidal proteins and their release into the environment were hypothesized to affect non-target organisms and ecosystem functions. In particular, effects might be strengthened through the simultaneous use of various *cry* genes, as contained in the stacked hybrid MON 89034 × MON 88017.

Currently, most of the risk assessment of Bt maize is based on single event hybrids that are equipped with only one insecticidal resistance. Influences on the soil biota and functions have been addressed in several studies dealing with the soil and plant associated microflora under Bt maize cultivation and the decomposition of the voluminous crop residues as an important ecological factor in nutrient cycling and carbon sequestration in soils (Mocali, 2011; Yanni et al., 2010). Analyses of the soil and root-associated microbial communities in Bt and non-Bt maize fields resulted in no specific or temporary effects of the transgenic plants (Barriuso et al., 2012; Miethling-Graff et al., 2010; Prischl et al., 2012). The structure of the communities was shown to be less affected by the transgenic

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hybrid and the released cry protein as compared to environmental factors such as plant age or site characteristics (Baumgarte and Tebbe, 2005; Tan et al., 2010). On the other hand, conflicting results have been reported on the crop chemistry and decomposition rate of the plant residues after harvest. While a range of previous studies displayed increased lignin contents (Masoero et al., 1999; Poerschmann et al., 2005; Saxena and Stotzky, 2001) and a slower decomposition rate of Bt maize (Castaldini et al., 2005; Dinel et al., 2003; Flores et al., 2005), several other studies did not reveal such changes of Bt maize under climate chamber and field conditions (Jung and Sheaffer, 2004; Mungai et al., 2005; Zurbrügg et al., 2010). It remains open if these contrasting results are due to the different plant material, growth conditions and methodological approaches.

The transformation of organic matter is essentially mediated by the activities and interactions of a multitude of soil microorganisms living in close relation to the plant residues. At present, relatively little research has been done in investigating the microbial communities involved in decomposition of residues from transgenic plants. Studies analysing cultivable soil bacteria and fungi displayed no significant or only short-term effects of the Bt-plant residues (Flores et al., 2005; Mulder et al., 2006). Minor, not lasting or not significant changes within the soil and residue colonizing microflora were also found in polyphasic approaches involving DNA-based DGGE (denaturating gradient gel electrophoresis) and T-RFLP (terminal restriction fragment length polyphormism) analyses targeting ribosomal RNA genes (Tan et al., 2010; Xue et al., 2011). Both, culture techniques combined with complementary methods and the different fingerprint techniques are suitable instruments to detect changes in the microbial communities (Smalla et al., 2007; van Elsas et al., 1998). However, analyses based on extracted sample DNA describe the total microbial community, which includes metabolically active groups as well as dormant and dead cells. Since soil microorganisms are thought to be in large part inactive (Olsen and Bakken, 1987) and DNA can persist in dead cells and as extracellular DNA in soils (Willerslev et al., 2004), these approaches do not necessarily reflect the microbial groups currently performing metabolic functions. Recently, RNA-based methods have been increasingly used to analyze metabolically active members of the microbial communities. The amount of rRNA per cell roughly correlates with the growth activity of bacteria (Molin and Givskov, 1999; Wagner, 1994) and allows the detection of living microorganisms constituting most of the metabolic activity. Though this approach has a couple of pitfalls, such as the extraction of RNA from soil (Sessitsch et al., 2002; Wang et al., 2012), varying ribosome contents per cell and the occurrence of RNA reserves in dormant cells (Sukenik et al., 2012), RNA based surveys represent a suitable strategy for revealing metabolically active microbial communities and detecting links to functional parameters such as organic matter decomposition.

Assuming that some of the plant compounds and their ratio are changed by the transgenic modification, the activity as well as the community structure of the decomposing microflora could be influenced. We combined the analysis of straw decomposition and the study of the decomposing microflora to evaluate the impact of the multi-resistant Bt maize hybrid MON 89034 × MON 88017. Our approach includes the quantification of the metabolically active microbial groups by taxon-specific primers, and the T-RFLP analyses of the residue colonizing bacterial and fungal communities.

2. Material and methods

2.1. Maize cultivars

Analyses were performed with the stacked Bt maize hybrid MON 89034 × MON 88017 (3-Bt), its near-isogenic control DKC 5143 (n-iso) which shares most of its genetic background with the transgenic hybrid, and the conventional varieties DKC 4250, Benicia, DKC 3420 and Agrano. MON 89034 × MON 88017 represents a F1 hybrid resulting from the hybridization of maize inbred MON 89034 with MON 88017. The transgenic hybrid produces the Cry1A.105 and Cry2Ab2 proteins, which are active against the European corn borer (*Ostrinia nubilalis*) and the Cry3Bb1 protein conferring resistance to corn rootworm (*Diabrotica virgifera*). Additionally, tolerance to glyphosate is provided by the expression of the *cp4 epsps* gene. Seeds of the maize varieties were obtained from Monsanto Agrar GmbH, Düsseldorf/Germany (MON 89034 × MON 88017, DKC 5143, DKC 4250), Pioneer Hi-Breed GmbH, Buxtehude/Germany (Benicia) and Saatbau Linz, Austria (DKC 3420, Agrano).

Table 1
Selected physico-chemical soil properties[a] of the field release experiment in Braunschweig (Germany). For each row of the field release experiment mean values and standard errors of mean were presented.

Field row	Soil texture			Total organic C (%)	pH-Value
	Sand (%)	Silt (%)	Clay (%)		
A	64.2 ± 2.7	30.3 ± 2.6	5.4 ± 0.6	2.4 ± 1.0	5.9 ± 0.06
B	57.3 ± 1.4	37.1 ± 1.3	5.6 ± 0.5	2.6 ± 0.9	5.9 ± 0.02
C	50.6 ± 1.8	42.2 ± 2.2	7.2 ± 1.7	2.5 ± 0.1	6.0 ± 0.05
D	46.7 ± 2.1	46.0 ± 1.5	7.2 ± 0.7	2.4 ± 0.1	6.0 ± 0.03
E	47.1 ± 2.0	45.6 ± 2.1	7.3 ± 0.3	2.4 ± 0.2	6.0 ± 0.04
Average	53.2 ± 7.0	40.2 ± 6.2	6.5 ± 1.2	2.5 ± 0.6	6.0 ± 0.06

[a] Data of the soil texture were obtained from Niemeyer et al. (pers. comm).

2.2. Field release experiment

Bt maize MON 89034 × MON 88017, the nontransgenic control DKC 5143 and the conventional varieties DKC 4250 and Benicia were continuously grown over three years in a field experiment on a loamy sand in Braunschweig, Lower Saxony (Dohrmann et al., 2013). Physico-chemical soil properties are indicated in Table 1. The plots (30 m × 42 m) were evenly distributed over five rows in eight replications. With a 3 m distance between the rows and an 8 m wide strip planted with maize (DKC 4250), the experiment covered an area of 6.7 ha. The maize varieties were uniformly supplied with fertilizers and herbicides according to the integrated crop management. At grain maturity (October) the plots were harvested by threshing, the straw was chopped roughly and spread over the soil surface.

2.3. Sample collection and preparation

Immediately after harvest, mixed straw samples (~300 g) containing stems, leaves and tassels were taken from each plot (15 sampling points per plot) and dried (60 °C) within 24 h to a constant weight. Then, the material was ground in an ultra-centrifugal mill with a vibratory feeder (ZM 200, Retsch Haan, Germany) to pass a 0.75-mm sieve and stored until used in the decomposition experiment.

Mixed samples of rotting straw (~300 g) were collected as described above four and eight weeks after harvest or during a frost-free period (January–March) and at the beginning of vegetation (April). Directly after sampling, the material was transported under cool conditions to the laboratory. Rotting straw without loosely adhering soil was taken with tweezers to get a subsample of 5 g which was ground in liquid nitrogen using mortar and pestle. Two aliquots of 150 mg each were stored at −80 °C until DNA and RNA extraction.