Pedobiologia **51** (2007) 195—206

Available online at www.sciencedirect.com
ScienceDirect

Pedobiologia
www.elsevier.de/pedobi

SOIL ECOLOGICAL AND ECONOMIC EVALUATION OF GENETICALLY MODIFIED CROPS — ECOGEN

Microbial and microfaunal community structure in cropping systems with genetically modified plants

Bryan S. Griffiths[a,*], Sandra Caul[a], Jacqueline Thompson[a], A. Nicholas E. Birch[a], Jérôme Cortet[b], Mathias N. Andersen[c], Paul Henning Krogh[d]

[a]*Environment Plant Interactions Programme, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK*
[b]*ENSAIA-INPL/INRA, UMR 1120, Laboratoire Sols et Environment, 2 Avenue de la forêt de Haye, BP 172, 54505 Vandoeuvre-lès-Nancy, France*
[c]*University of Aarhus, Faculty of Agricultural Sciences, Research Centre Foulum, PO Box 50, DK-8830 Tjele, Denmark*
[d]*Soil Fauna and Ecotoxicology Research Unit, Department of Terrestrial Ecology, National Environmental Research Institute, PO Box 314, Vejlsoevej 25, DK-8600 Silkeborg, Denmark*

Received 29 March 2007; accepted 10 April 2007

KEYWORDS
Bt-maize;
Genetically modified crops;
Herbicide tolerant maize;
Microbial community structure;
Nematodes;
Protozoa

Summary

Soils from field sites at Foulum (DK), Narbons (FR) and Varois (FR) planted with genetically modified maize expressing either the insecticidal *Bacillus thuringiensis* protein (*Bt*) or herbicide tolerance (HT), as described elsewhere in this volume, were analysed for nematodes, protozoa and microbial community structure. These analyses were mirrored in single-species testing and in mesocosm experiments, and were coordinated with field samples taken for microarthropods, enchytraeids and earthworms so allowing for cross-comparison and a better understanding of the results observed in the field. Over the first 2 years of the field experiments (in 2002 and 2003), the effect of *Bt*-maize was within the normal variation expected in these agricultural systems. Sampling in 2004 and 2005 was expanded to include the effects of tillage (i.e. reduced tillage versus conventional tillage) and also the use of HT-maize. Tillage had major effects regardless of soil type (Varois or Foulum), with reduced-tillage plots having a greater abundance of microfauna and a different microbial community structure (measured both by phospholipid fatty-acid analysis (PLFA) and by community-level physiological profiling (CLPP)) from conventionally tilled plots. Grass, as a contrasting cropping system to maize, also had an effect regardless of soil type and resulted in greater microfaunal abundance and an altered microbial community structure. Differences in crop management, which for the *Bt*-maize was removal of the insecticide used to control European corn borer and for HT-maize was a change in herbicide formulation, were only tested at single sites. There were differences in microbial community structure (CLPP but not PLFA) and

*Corresponding author. Tel.: +44 1382 562731; fax: +44 1382 568502.
E-mail address: bryan.griffiths@scri.ac.uk (B.S. Griffiths).

0031-4056/$ - see front matter
doi:10.1016/j.pedobi.2007.04.002

sporadic increases in protozoan abundance under the *Bt*-crop management. The HT-maize cropping system, which covered a shorter period and only one site, showed little change from the conventional system other than an altered microbial community structure (as measured by PLFA only) at the final harvest. The *Bt*-trait had a minimal impact, with fewer amoebae at Foulum in May 2003, fewer nematodes at Foulum in May 2004 but more protozoa at Varois in October 2002 and an altered microbial community structure (PLFA) at Foulum in August 2005. These were not persistent effects and could not be distinguished from varietal effects. Based on the field evaluations of microfauna and microorganisms, we conclude that there were no soil ecological consequences for these communities associated with the use of *Bt*- or HT-maize in place of conventional varieties. Other land management options, such as tillage, crop type and pest management regime, had significantly larger effects on the biology of the soil than the type of maize grown.

Introduction

Despite the large area of *Bt*-maize (i.e. genetically modified (GM) maize expressing an insecticidal protein from *Bacillus thuringiensis*) and other *Bt* crops planted, there are still scientifically interesting questions to be addressed with regard to the little-known soil compartment (see, for example, reviews by Groot and Dicke, 2002; Bruinsma et al., 2003; Dunfield and Germida, 2004; Motavalli et al., 2004; Stotzky, 2004; O'Callaghan et al., 2005). The same is true of herbicide-tolerant (HT) crops, with few studies related to soil ecological effects. A practical consequence of the introduction of these (i.e. *Bt* and HT) GM crops is the likely modification of the cropping system to maximize the benefits associated with the technology. Thus, for GM plants expressing the *Bt* protein this includes the reduced application of insecticide and for HT-crops the likely conversion to reduced-tillage operations. In a study of the impacts of GM plants on soil populations and processes, it is relevant, therefore, to compare not only the GM and conventional cultivars but also the likely cropping systems in which these GM plants would be grown. To address these issues the European Commission-funded ECOGEN project (www.ecogen.dk) was initiated. Initial results from the ECOGEN project revealed minimal differences between *Bt* and conventional cultivars. Results after the first 2 years of field trials with Cry1Ab-expressing maize showed that changes to microbial and microfaunal (protozoan and nematode) communities due to the *Bt* trait were small and less than changes due to different (non-*Bt*) maize cultivars and different crops (Griffiths et al., 2005). Decomposition of wheat straw in the same field trials was unaffected by *Bt*-maize (Cortet et al., 2005). A similar conclusion was drawn from a pot experiment, that although there are effects of the *Bt* trait on soil microbial and faunal communities, they are relatively small compared with effects of soil type (field site) and maybe confounded by natural variation between different maize lines (Griffiths et al., 2006, 2007). To expand these findings into a comparison of cropping systems including GM cultivars, the field trials (described elsewhere in this volume, Andersen et al., 2007) were modified to include the likely cropping-system changes associated with the production of GM maize, namely, reduced tillage and no insecticide application to control European corn borer (*Ostrinia nubilalis* Hübner). These different management practices had the additional benefit of providing a set of internal positive controls against which to compare differences due to the cultivars planted (either conventional or GM). As the same field plots were used as previously reported on (Griffiths et al., 2005), the data presented here also represent the final 2 years of 4-year continuous *Bt*-maize production at the Foulum site.

Materials and methods

Field sites

The field sites at Foulum (Northern Jutland, Denmark), Varois (Bourgogne, France) and Narbons (Midi-Pyrenées, France) are described in detail by Andersen et al. (2007, this volume) and were sampled during 2004 and 2005. Briefly, at Foulum different maize varieties were grown in a four-block, split-plot design, with each plot divided into a conventionally tilled part and a reduced-tillage part. The maize varieties were MEB307 (a MON810 *Bt*-variety, from Monsanto); Monumental (the conventional variety isogenic to MEB307 but without

the *Bt*-trait); DK242 (another conventional variety, but not sampled in this study); and N32-K3 (a glufosinate-ammonium (Basta)-tolerant variety, from Syngenta). At Varois, the *Bt*-variety DKc3946 and the conventional variety DK315 were combined with conventional (ploughing, harrow and rotary harrow) versus reduced tillage (only harrow and rotary harrow) in a four-block, split-plot design where tillage was the main-plot factor and variety was the subplot factor. At Narbons, treatments were placed in a four-block design with completely randomized plots in which maize varieties, suited for the regional climate, were planted. Three maize varieties and two pesticide regimes were used at Narbons: DKc5784 (MON810 *Bt*-variety, from Monsanto) treated with pesticides suitable for *Bt*-maize (PAS *Bt*); DKc5783 (a conventional variety near-isogenic to DKc5784 but without the *Bt*-trait) treated with the same pesticide regime suitable for *Bt*-maize (PAS *Bt*); DKc5783 treated with pesticides suitable for non-*Bt* maize (PAS Con.); and Paolis (a registered conventional maize variety) that was treated with pesticides suitable for non-*Bt* maize (PAS Con.). The plots at Narbons were irrigated regularly throughout the growing season and each plot was separated by grass sown when the plots were established in 2003 (new grass), while the outer perimeter of the plots was surrounded with mature grass (old grass).

Soil sampling

Soil samples were collected in May and October 2004 and 2005 (all sites) and additionally in August 2005 from Foulum and Narbons only. Samples from the top 15 cm were analysed as described previously (Griffiths et al., 2005) for gravimetric water content; nematode abundance (Whitehead and Hemming, 1965; samples from Foulum and Varois were then further processed through glycerol and mounted on a glass slide for taxonomic identification at higher magnification); protozoa by a most probable number technique (Darbyshire et al., 1974); microbial community-level physiological profile (CLPP, Garland and Mills, 1991); and phospholipid fatty acid analysis (PLFA in 2004) and ester-linked fatty acid analysis (ELFA in 2005). For PLFA, total lipids were extracted from the frozen aliquots of soil with citrate buffer, methanol and chloroform (Nielsen and Petersen, 2000), PLFAs were then separated by solid-phase extraction, converted to methyl esters by mild alkaline transesterification and analysed by gas chromatography using a low-polarity column (Frostegård et al., 1991). For ELFA, the frozen aliquots of soil were extracted with KOH in methanol, neutralized with acetic acid, fatty acid methyl esters were extracted with hexane (Schutter and Dick, 2000) and analysed by gas chromatography as for PLFA.

Statistical analysis

Data were analysed using standard analysis of variance (ANOVA) procedures with the Rothamsted GENSTAT programme (The GenStat Committee, 2005) and presented as means with an associated least significant difference (lsd, at the 5% level) or an associated standard deviation. Protozoan abundances (natural logarithm) and proportions of nematode trophic groups (angular) were transformed prior to analysis to normalize the data; back-transformed means are used throughout the text and tables. The time-course profiles of the CLPP data were analysed from the area under the colour development profile (Hackett and Griffiths, 1997). The results of the CLPP time course, the PLFA and ELFA profiles, as well as the taxonomic composition of the nematode communities were analysed by principal component (PC) analysis and the resulting PC scores were analysed by ANOVA.

Results

Foulum

There were consistently greater abundances of nematodes (Table 1), microbial biomass and a bacterial:fungal ratio (Table 2) in the reduced tillage than in the conventionally tilled plots, and a consistently different microbial community structure as measured by PC analysis of the fatty acid profile (Table 3). Tillage inconsistently affected soil water content, with soil from the reduced-tillage plots being wetter than that from the conventional plots in October 2004, May 2005 and October 2005, and soil protozoa, with a greater abundance of amoebae and flagellates in the reduced-tillage plots in May 2005 (Table 1). There were no treatment effects on ciliate protozoa (grand mean $40\,\mathrm{ng\,g^{-1}} \pm 13$), nor tillage effects on the CLPP (Table 3). There was a reduced abundance of nematodes in the *Bt*-maize (MEB307) compared with near-isogenic, non-*Bt* maize (Monumental) plots in October 2005 (Table 1). Nematode community structure, analysed in the October 2004 and October 2005 samples, changed significantly with year, tillage and maize cultivar (Table 4, Fig. 1). Proportions of nematodes were significantly ($P < 0.05$) lower for bacterial feeders in 2005 than

Table 1. Per cent gravimetric soil water content (H_2O), numbers of nematodes g^{-1} (Nem), biomasses ($ng\,g^{-1}$) of amoebae (Amo) and flagellates (Fla) from three field sites under *Bt*-maize (MEB307, DKc3946 and DKc5784), near-isogenic non-*Bt*-maize (Monumental, DK315 and DKc5783), conventional maize (Paolis), HT-maize and recently established (new) or long-term (old) grass

Site	Treatment	2004								2005											
Crop		May				Oct				May				Aug				Oct			
		H_2O	Nem	Amo	Fla	H_2O	Nem	Amo	Fla	H_2O	Nem	Amo	Fla	H_2O	Nem	Amo	Fla	H_2O	Nem	Amo	Fla
Foulum																					
HT-maize	RT	21.68	31.4	780	279	27.62	27.02	2769	1075	24.4	17.06	915	473	20.7	17.49	2071	477	18.26	32.7	553	346
MEB307	RT	21.86	24.6	754	326	29.15	32.51	3754	1276	26.24	22.25	965	495	22.91	19.56	1200	412	18.86	29.1	983	172
Monumental	RT	21.95	30.3	923	445	28.2	30.63	2249	1454	25.2	16.69	467	309	20.65	16.12	1390	341	17.14	39.3	777	278
HT-maize	CT	21.09	20	411	212	26.41	17.28	6642	883	23.21	14.05	531	268	20.04	11.64	815	404	18.86	29.3	534	265
MEB307	CT	21.01	18.5	743	383	27.88	19.62	814	1119	24.77	13.67	324	208	21.96	10.85	665	330	18.69	22.2	275	225
Monumental	CT	21.99	27.1	267	292	28.87	20.01	2521	544	24.82	14.83	366	232	22.12	13.21	701	294	18.86	30.2	731	258
Lsd[a]	Crop	0.85	5	524	125	**0.65***	4.4	5401	4570	**0.65***	4.3	369	147	**0.86***	3.4	841	171	0.85	**6.2***	466	109
Lsd	Tillage	0.69	**4.1***	428	102	**0.53***	**3.6***	4410	3732	**0.53***	**3.5***	**301***	**120***	0.7	**2.8***	**686***	140	**0.70***	**5.0***	381	89
Lsd	C∗T	1.2	7.1	741	178	**0.93***	6.2	7638	6464	0.91	6	521	208	1.21	4.8	1189	242	1.21	8.7	659	154
Varois																					
DKc3946	RT	10.92	9.27	973	119	12.32	10.07	1432	311	16.46	16.19	1496	136	nd[b]	nd	nd	nd	15.76	50.8	1730	184
DK315	RT	10.7	7.63	228	56	12.61	8.03	1550	293	15.54	13.12	1834	201	nd	nd	nd	nd	16.05	44.4	815	191
DKc3946	CT	10.97	5.56	643	156	12.34	6.48	1066	71	15.03	10.73	393	77	nd	nd	nd	nd	16	13.4	460	94
DK315	CT	11.49	5.73	774	153	12.9	8.13	1735	525	14.45	9.79	686	144	nd	nd	nd	nd	15.35	11.5	715	90
Lsd	Crop	0.52	2	652	95	0.61	1.8	1171	11293	1.13	3.6	9.6	**53***	nd	nd	nd	nd	0.95	13.3	556	77
Lsd	Tillage	0.52	**2.0***	652	95	0.61	1.8	1171	11293	**1.13***	**3.6***	**9.6***	**53***	nd	nd	nd	nd	0.95	**13.3***	**556***	**77***
Lsd	C∗T	0.73	2.9	923	134	0.87	**2.5***	1656	15970	1.6	4.9	1296	75	nd	nd	nd	nd	1.34	18.8	**786***	109
Narbons																					
DKc5784	*Bt*	16.5	14.39	1404	225	15.75	10	606	55	16.05	4.37	900	71	22.28	5.20	3209	303	21.27	6.70	1295	76
DKc5783	Con	16.8	15.58	671	168	14.02	15.3	687	111	15.21	3.27	1215	107	21.32	6.20	1499	135	21.02	9.30	722	71
DKc5783	*Bt*	15.88	15.35	1550	100	13.92	10.7	375	65	16.2	3.66	1514	91	21.90	6.20	3307	334	21.47	8.00	429	123
Paolis	Con	16.86	15.71	1118	192	15.76	15.6	668	49	16.74	4.14	1242	67	21.69	5.30	2248	241	21.02	8.20	1496	146
New grass	Con	15.02	13.02	1030	188	15.5	20.9	2105	96	15.59	5.25	1334	96	22.67	10.80	1473	101	21.31	27.00	3448	280
Old grass	Con	15.89	15.07	2285	278	16.84	37.8	1021	149	11.77	11.22	1273	162	24.53	42.00	4950	269	21.53	38.10	1754	263
Lsd	Crop	1.32	**1.81***	1540	172	2.9	**10.1***	1316	92	**1.95***	**2.2***	1255	89	**1.27***	**7.6***	2516	183	0.90	**7.0***	2120	**127***

Plots at Foulum and Varois were treated with reduced (RT) or conventional tillage (CT), while plots at Narbons were treated either with conventional (Con) or *Bt* insect pest management.
*Significant differences within the site on the sampling occasion.
[a]Least significant difference, $P<0.05$, in bold with effects due to crop, tillage or a crop∗tillage interaction.
[b]Not determined.

Table 2. Microbial biomass (MB, nmol P g^{-1}, from phospholipid-linked, PLFA, or ester-linked, ELFA, fatty acids) and bacterial:fungal ratio from field sites as described in Table 1

Site	Treatment	2004				2005					
Crop		May		Oct		May		Aug		Oct	
		MB-PLFA	B:F ratio	MB-PLFA	B:F ratio	MB-ELFA	B:F ratio	MB-ELFA	B:F ratio	MB-ELFA	B:F ratio
Foulum											
HT-maize	RT	44.2	23.65	44.6	16.15	131	7.74	130.7	7.25	139.6	7.10
MEB307	RT	43.8	23.52	47.8	19.05	139	7.72	126.5	8.41	130.7	7.28
Monumental	RT	47.6	22.71	49.7	17.76	135.7	7.39	129.8	6.97	140.2	7.16
HT-maize	CT	41.4	29.74	34.5	22.59	111.8	9.18	114.2	8.01	125.6	8.98
MEB307	CT	44.6	30.80	42.8	25.84	121.3	10.15	115.3	9.67	123.7	9.80
Monumental	CT	46	28.93	40	27.70	122.4	9.91	115.5	10.12	121.3	9.60
Lsd[a]	Crop	7.89	4.84	10.68	4.47	9.36	1.09	9.97	1.22	8.65	0.99
Lsd	Tillage	6.44	**3.95***	8.72	**3.65***	**7.66***	**0.89***	**8.14***	**1.00***	**7.06***	**0.80***
Lsd	C∗T	11.16	6.84	15.11	6.32	13.26	1.55	14.1	1.73	12.23	1.4
Varois											
DKc3946	RT	27.5	27.00	55.4	14.70	127.30	3.80	nd[b]	nd	119.60	3.07
DK315	RT	22.9	25.90	45.6	14.10	133.20	3.65	nd	nd	119.60	3.22
DKc3946	CT	29.2	23.90	51.2	12.20	82.60	5.11	nd	nd	93.50	5.08
DK315	CT	25.7	24.60	48.5	17.00	78.20	4.74	nd	nd	88.90	4.76
Lsd	Crop	5.5	6.76	8.14	5.45	8.36	1.01	nd	nd	4.64	0.93
Lsd	Tillage	5.5	6.76	8.14	5.45	**8.36***	**1.01***	nd	nd	**4.64***	**0.93***
Lsd	C∗T	7.78	9.57	11.52	7.71	11.83	1.428	nd	nd	6.56	1.32
Narbons											
DKc5784	*Bt*	35	20.14	42.83	22.26	101.6	6.08	106	4.90	120.4	5.84
DKc5783	Con	34.3	20.86	44.31	18.07	99.4	5.97	109.7	4.98	113.7	6.02
DKc5783	*Bt*	32.9	24.78	42.08	24.01	94	5.58	100.7	5.25	144.4	4.82
Paolis	Con	30.2	20.95	43.19	24.30	96.2	5.84	109.9	4.35	117.1	6.15
New grass	Con	32.2	22.85	47.76	9.62	226.7	6.71	245.9	4.33	130.5	3.95
Old grass	Con	74.6	8.32	78.08	9.41	92.4	5.58	102.2	5.66	236.1	4.49
Lsd	Crop	**12.18***	**5.86***	**8.39***	**4.96***	**31.34***	1.27	**27.84***	1.30	**31.71***	1.03*

*Significant differences within the site on the sampling occasion.
[a]Least significant difference, $P<0.05$, in bold with effects due to crop, tillage or a crop∗tillage interaction.
[b]Not determined.

in 2004, greater ($P<0.001$) for fungal feeders with reduced tillage than with conventional tillage, greater ($P<0.001$) for omnivores in 2004, and lower ($P<0.001$) for plant feeders with reduced tillage. This was mirrored in the PC analysis, which revealed significant effects in PC 1 of year ($P<0.001$) and tillage ($P<0.05$), in PC 2 of tillage ($P<0.001$) and in PC 4 of tillage ($P<0.05$) and treatment ($P<0.001$) (Fig. 1). The latent vector loadings indicated that the nematode taxa contributing to the differences were: for PC1, positively – *Wilsonema*, *Helicotylenchus*, negatively – *Pratylenchus*, Dolichodoridae, *Teratocephalus*; for PC 2, positively – Tylenchidae, Aphelenchoididae, negatively – Rhabditidae, *Pratylenchus*, *Helicotylenchus*; for PC4, positively – Monhysteridae, Dorylaimidae, *Acrobeles*, negatively – *Paratylenchus*, *Eucephalobus*, *Helicotylenchus*. The treatment effect in PC 4 was because of HT-maize, with no significant ($P>0.05$) difference between *Bt* and non-*Bt* maize. Soil collected from the HT-maize plots had a significantly different microbial community structure (ELFA) in August 2005 (Table 3), and was also drier in October 2004, May and August 2005 (Table 1) than under the other treatments.

Varois

There were no consistent effects of any of the treatments on the variables measured. However, the reduced-tillage plots tended to differ significantly from the conventionally tilled plots in 2005 and not in 2004 (e.g. soil water content, nematode and protozoan abundance (Table 1), microbial

Table 3. Principal component (PC) scores from analyses of microbial community structure by community-level physiological profiles (CLPP), phospholipid-linked fatty acids (PLFA) or ester-linked fatty acids (ELFA), from field sites as described in Table 1

Site Crop	Treatment	CLPP 2004 May PC1	PC2	Oct PC1	PC2	2005 May PC1	PC2	Aug PC1	PC2	Oct PC1	PC2	PLFA 2004 May PC1	PC2	Oct PC1	PC2	ELFA 2005 May PC1	PC2	Aug PC1	PC2	Oct PC1	PC2
Foulum																					
HT-maize	RT	−26.0	−2.6	−0.2	−0.8	−4.1	−0.5	−0.4	2.2	−1.3	−4.0	1.4	0.4	−1.1	0.9	1.1	0.1	1.3	−0.2	1.4	0.0
MEB307	RT	17.0	−27.9	−0.8	2.2	−1.7	0.2	−1.3	0.6	0.6	3.8	0.2	0.5	0.6	0.6	1.2	0.3	0.1	−0.3	0.9	0.1
Monumental	RT	0.0	9.1	−1.8	−1.7	4.9	−0.4	−0.4	1.1	2.2	−0.6	0.3	0.6	−0.5	0.8	1.6	−0.1	1.7	0.0	1.4	0.3
HT-maize	CT	22.0	−5.0	−0.9	−1.1	1.5	1.8	1.8	−0.7	−3.9	0.8	−0.6	−0.6	−1.3	−1.0	−1.0	−0.4	0.3	−0.2	−0.8	−0.3
MEB307	CT	−33.0	−1.0	4.8	−1.6	−1.8	2.5	−1.7	−1.6	0.9	−1.3	−0.9	−0.5	1.1	−0.6	−1.4	0.1	−1.4	0.4	−1.4	0.0
Monumental	CT	19.0	27.6	−1.1	3.0	1.1	−3.6	2.0	−1.6	1.5	1.4	−0.5	−0.5	1.2	−0.7	−1.5	0.0	−2.0	0.2	−1.4	0.0
Lsd[a]	Crop	63.40	38.74	5.50	3.88	5.86	4.32	6.04	4.56	5.04	3.29	1.43	0.51	2.16	0.93	0.80	0.49	**1.16***	0.73	0.56	0.47
Lsd	Tillage	51.80	31.62	4.50	3.17	4.78	3.53	4.94	3.72	4.12	2.68	**1.17***	**0.42***	1.77	**0.76***	**0.65***	0.40	**0.94***	0.59	**0.46***	0.38
Lsd	C∗T	89.80	54.78	7.80	5.48	8.30	6.11	8.54	6.45	7.14	**4.68***	2.03	0.73	3.06	1.32	1.13	0.69	1.63	1.03	0.79	0.66
Varois																					
DK315	RT	2.4	−1.2	−1.6	−0.2	−4.4	−0.3	nd[b]	nd	0.3	0.4	−0.8	0.3	−0.5	−0.2	3.1	−1.0	nd	nd	−2.4	−0.4
DKc3946	RT	0.6	0.6	−1.6	−1.2	2.9	0.2	nd	nd	0.9	−0.9	0.8	0.6	−0.7	0.8	2.3	−1.0	nd	nd	−2.3	−0.1
DK315	CT	−1.0	1.1	1.7	−0.3	0.8	−2.0	nd	nd	−0.3	0.1	0.1	0.1	1.2	0.0	−3.5	−0.5	nd	nd	2.1	0.3
DKc3946	CT	−2.0	−0.5	1.5	1.7	0.6	2.1	nd	nd	−0.9	0.4	−0.1	−1.0	0.0	−0.6	−3.7	−0.5	nd	nd	2.6	0.3
Lsd	Crop	5.38	3.31	5.58	4.34	7.12	4.02	nd	nd	4.16	2.17	1.54	1.04	1.52	1.29	1.75	0.87	nd	nd	2.43	1.18
Lsd	Tillage	5.38	3.31	5.58	4.34	7.12	4.02	nd	nd	4.16	2.17	1.54	1.04	1.52	1.29	**1.75***	0.87	nd	nd	**2.43***	1.18
Lsd	C∗T	7.62	4.69	7.90	6.14	10.06	5.68	nd	nd	5.88	3.07	2.18	1.47	2.14	1.83	2.47	1.23	nd	nd	3.44	1.67
Narbons																					
DKc5784	*Bt*	1.8	2.1	1.8	−0.3	0.2	−0.4	0.4	1.8	−1.2	−1.5	−0.8	0.3	−1.8	−0.2	−1.0	0.1	−0.9	0.8	−2.0	0.4
DKc5783	Con	−2.8	−5.9	1.9	−3.7	−2.9	0.3	2.1	−0.1	2.4	−1.2	−1.0	0.1	−0.9	0.4	−1.2	0.2	−0.6	0.6	−1.6	0.3
DKc5783	*Bt*	−3.7	−2.0	3.2	0.9	3.5	0.7	0.8	−0.5	0.8	1.1	−1.3	0.1	−1.6	−0.1	−0.9	0.2	2.6	0.6	0.5	−0.1
Paolis	Con	1.3	5.6	2.7	−1.3	−4.4	0.3	−1.4	−0.8	−3.8	2.2	−0.9	0.0	−2.0	−0.5	−0.7	−0.2	−0.8	0.6	−1.5	0.8
New grass	Con	6.8	3.3	−6.5	1.4	2.7	−1.0	−1.4	1.4	−1.7	0.8	−0.7	−0.6	3.6	−1.1	−0.4	1.2	0.1	0.7	1.2	−2.9
Old grass	Con	−3.5	−3.1	−3.1	2.9	0.9	0.2	−0.5	−1.7	3.5	−1.3	4.7	0.1	2.8	1.6	4.3	−1.5	−0.5	−3.3	3.3	1.5
Lsd	Crop	8.26	8.68	10.00	7.74	6.90	4.81	5.22	3.65	5.70	5.08	**1.41***	1.37	**1.88***	**1.63***	**3.43***	1.67	4.62	**1.80***	**2.97***	**1.34***

*Significant differences within the site on the sampling occasion.

[a]Least significant difference, $P<0.05$, in bold with effects due to crop, tillage or a crop∗tillage interaction.

[b]Not determined.

Table 4. Per cent contribution of bacterial-feeding (BF), fungal-feeding (FF), omnivorous (OM) and plant-feeding (PF) nematodes, from Foulum and Varois in October as described in Table 1

Site	Crop	Treatment	Nematode trophic group			
Year			BF	FF	OM	PF
Foulum						
2004	HT-maize	RT	49.2	25.9	5.9	17.8
	MEB307	RT	39.9	36.7	6.9	13.1
	Monumental	RT	44.9	33.3	3.7	16.3
	HT-maize	CT	47.0	26.5	4.1	20.4
	MEB307	CT	41.5	25.2	6.8	25.5
	Monumental	CT	44.9	22.6	5.6	25.3
2005	HT-maize	RT	42.2	26.6	3.1	25.6
	MEB307	RT	38.1	35.9	0.8	22.9
	Monumental	RT	37.0	37.6	3.1	20.2
	HT-maize	CT	40.9	21.0	1.4	34.4
	MEB307	CT	37.5	24.7	0.9	35.1
	Monumental	CT	36.7	20.9	2.5	36.7
Lsd[a]	Year		**0.048***	0.045	**0.038***	**0.048***
Lsd	Treatment		0.048	**0.045***	0.038	**0.048***
Lsd	Crop∗year		0.082	0.078	**0.065***	0.084
Varois						
2004	DKc3946 *Bt*	RT	36.1	52.1	2.5	6.3
	DK315 non-*Bt*	RT	38.7	51.0	4.1	2.4
	DKc3946 *Bt*	CT	47.2	34.8	5.6	7.8
	DK315 non-*Bt*	CT	42.8	46.5	4.5	2.6
2005	DKc3946 *Bt*	RT	36.7	58.9	0.1	3.3
	DK315 non-*Bt*	RT	33.3	63.4	0.5	1.2
	DKc3946 *Bt*	CT	51.0	43.9	0.6	1.9
	DK315 non-*Bt*	CT	39.9	56.0	0.7	0.5
Lsd	Year		0.062	**0.058***	**0.045***	**0.061***
Lsd	Crop		0.062	**0.058***	0.045	**0.061***
Lsd	Treatment		**0.062***	**0.058***	0.045	0.061

*Significant differences within the site on the sampling occasion.
[a]Least significant difference, $P<0.05$, in bold of means given angular transformation (means in table are back-transformed), with effects due to crop, tillage or a crop∗tillage interaction.

biomass (Table 2) and microbial community structure by fatty acid analysis (Table 3)). There were no treatment effects on ciliate protozoa (grand mean 64 ng $g^{-1} \pm 16$). Nematode community structure, analysed from the October 2004 and October 2005 samples, changed significantly with year, tillage and maize cultivar in some cases (Table 4, Fig. 1). Proportions of nematodes were significantly ($P<0.01$) lower for bacterial feeders with reduced tillage than with conventional tillage, greater ($P<0.001$) for fungal feeders with reduced tillage than with conventional tillage and also greater ($P<0.05$) under *Bt*-maize than under non-*Bt* maize, but only in 2004, greater ($P<0.001$) for omnivores in 2004 than in 2005, greater ($P<0.005$) for plant feeders in 2005 and also greater ($P<0.005$) under *Bt*-maize than under non-*Bt* maize. The PC analysis (Fig. 1) revealed that nematode community structure varied in a complex way, with effects seen in PC 1 because of tillage ($P<0.001$) and year ($P<0.001$) with a tillage∗crop∗year interaction ($P<0.05$); PC 2 also revealed effects of tillage ($P<0.001$) and year ($P<0.001$), with a tillage∗crop interaction ($P<0.01$) and PC 3 showed a year effect ($P<0.01$). The interpretation of Fig. 1 is that there was a difference due to tillage only in 2005 and not in 2004, while *Bt* induced a different nematode community structure only in 2004 in the reduced-tillage treatment. The latent vector loadings indicated that the nematode taxa contributing to the differences were: for PC1, positively – Aphelenchoididae, *Eucephalobus*, negatively – Rhabditidae, Tylenchidae; for PC 2, positively – Tylenchidae, *Eucephalobus*, Dorylaimidae, negatively – Rhabditidae, Aphelenchoididae; for PC3, positively –Tylenchidae, Rhabditidae, Aphelenchoididae, negatively – *Acrobeloides*.

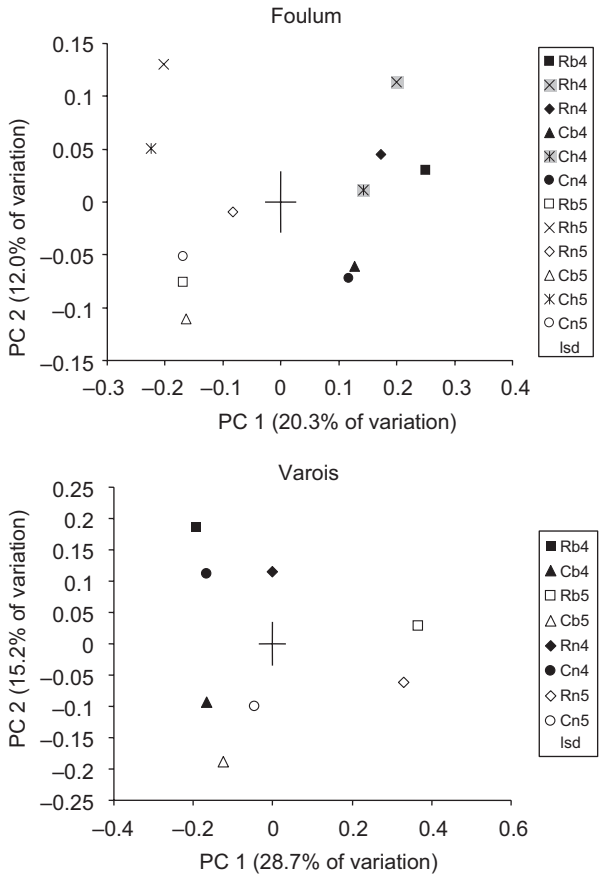

Figure 1. Principal component (PC) plot of nematode community structure (based on taxonomic identification) at Foulum and Varois, from reduced and conventionally tilled plots (symbols with 'R' and 'C' suffixes, respectively), planted with *Bt*-, non-*Bt* or HT-maize ('b', 'n' and 'h'), in October 2004 and in October 2005 ('4' and '5'). Bars represent the overall least significant difference, $P<0.05$.

Narbons

There was a consistent difference between the grass plots and the maize plots, with a greater abundance of nematodes (Table 1) and microbial biomass (Table 2) and altered microbial community structure by fatty acid analysis (Table 3). Soil from under grass also differed in water content (May and August 2005) and had a greater abundance of flagellates than the maize plots in October 2005 (Table 1). There were no significant differences in the soil biota assessed between the maize cultivars or crop management regimes.

Discussion

Bt-maize has been the subject of more soil-based studies than HT-maize (see reviews by Groot and Dicke, 2002; Bruinsma et al., 2003; Dunfield and Germida, 2004; Motavalli et al., 2004; Stotzky, 2004; Liu et al., 2005; Clark et al., 2005; O'Callaghan et al., 2005). The UK-based farm-scale evaluation (FSE) comparing cropping systems with conventional or HT-crops was concerned mainly with above-ground organisms (Firbank et al., 2003), so the study reported here is complementary to those detailed measurements. The difference in emphasis towards these two GM traits is probably because conceptually plants transformed to express the *Bt* insecticidal protein produce a compound that is toxic to some organisms, so there is a greater perception of potential side effects to non-target organisms than there is with GM HT-plants whose product is not purposely toxic to some organisms. Another perception is that while any difference between *Bt*- and non-*Bt* plants could be related back to the genetic difference between the cultivars, differences between HT- and non-HT-plants would be related back to differences in herbicide application (i.e. not directly related to the genetic modification). However, at the cropping system level, the utilisation of *Bt*-plants would involve a change in insecticide application and so have indirect effects in a similar way to HT-plants. Also, the expressed transgene product from GM HT-plants will be exuded from the roots and contained in plant debris, with the same opportunities for interaction with soil organisms as the *Bt* protein (Dunfield and Germida, 2004).

The main influences affecting the soil populations in this study were those of tillage; crop type (grass or maize); location (soil type and climate) and season. Differences due to the *Bt*- and the HT-traits were inconsistent. There were significantly fewer nematodes under *Bt*-maize than under non-*Bt* maize at Foulum, but only in October 2005 and not in the other sampling occasions, with no significant differences in nematode abundance at Varois or Narbons. In the first 2 years of the study (Griffiths et al., 2005), there was only a statistically detectable difference in nematode abundance between *Bt*- and non-*Bt* maize when data from all three sites were pooled. Protozoa, which exhibited some transient differences between *Bt*- and non-*Bt* maize previously (Griffiths et al., 2005), did not differ as a consequence of the *Bt*-trait at any of the sites in this study. The difference between the initial effects and the ones seen in this study could reflect the normal temporal variation from season to season or it may be that populations are stabilizing to the maize monoculture after the transition from the previous cropping system. Seasonal differences are exemplified in the example that 2005 was wetter than 2004 at Varois and

this coincided with bigger differences between tillage treatments. The presence of seasonal and climatic variation is a major difference between field and pot studies, something that is explored in other papers in this volume (Birch et al., 2007, this volume). From these studies at three sites over 4 years with a variety of cropping scenarios, we can confidently state that *Bt*-maize had no effects on soil microfauna and microorganisms greater than seasonal, soil, tillage, cultivar and crop type (maize or grass) effects. The study of HT-maize covered a shorter period and only one site, but we also saw no lasting differences in soil populations attributable to the HT-cropping system. Studies have shown that soil faunal populations can take several years to reach equilibrium following changes in land management (Yeates et al., 1999), so the results from this study may only be relevant for the early stages of the transition to the new management regime.

Tillage proved to have the single most significant effects on soil populations. In an extensive review of the impacts of disturbance through tillage on food webs in agro-ecosystems, Wardle (1995) concluded that tillage tends to reduce large soil organisms (beetles, spiders, earthworms) more than the smallest ones (bacteria, fungi), with some intermediate groups such as bacterial-feeding nematodes, mites and enchytraeids even showing small population increases. A later review (Holland, 2004), however, concluded that microorganisms, meso- and macro-fauna were all stimulated by conservation (reduced) tillage. For most species groups, the effects on populations were due to indirect effects arising as a result of the modification of soil habitats, particularly the continuity of water-filled pores and water films (Winter et al., 1990). Smaller impacts of changes in tillage practice are therefore often seen on very sandy soils (Spedding et al., 2004). We did not detect this type of difference between the sandier Foulum soil and the clay-rich Varois soil, with the former soil exhibiting the more consistent responses to tillage. It is often predicted that fungal populations will be reduced more significantly than bacterial populations by tillage due to the disruption of the hyphal network (Doran and Linn, 1994). However, Wardle (1995) showed that under similar practices, bacterial and fungal populations are reduced slightly and to the same extent by tillage in annual cropping systems. Petersen et al. (2002) showed that despite slight differences in microbial biomass between no-till and ploughed systems, the seasonal dynamic interactions of soil conditions and microbiological properties were similar, suggesting that common mechanisms regulate microbial dynamics in both tillage systems. Ahl et al. (1998) showed that reduced-tillage methods tended to increase the total amount and proportion of fungal biomass in soil relative to bacteria. The results from the tillage treatments at Foulum and Varois consistently revealed increases in microbial and faunal biomass, with a shift towards fungally dominated systems as indicated by the fungal fatty acid markers and fungal-feeding nematodes. This was unaffected by the inclusion of the GM crops in the system.

In previous studies, the CLPP analysis proved to be more sensitive than the PLFA analysis, in that it would detect differences between treatments where the latter would not (Griffiths et al., 1999, 2005; Bruinsma et al., 2002). This has been interpreted as PLFA revealing more fundamental, structural changes in microbial community structure, with CLPP incorporating differences in function. Given that the effects on PLFA seen at Foulum and Varois were generally a result of tillage and that largely due to a shift towards fungally dominated systems under minimum tillage, it could be that the CLPP, which is strictly a measure of bacterial activity, would not be responsive to the tillage treatments. There were no differences seen previously in the CLPP or the PLFA between MEB307 and monumental at Foulum or between maize cultivars at Varois (Griffiths et al., 2005), which mirrors the results of this study. The only statistically significant effects in CLPP at Foulum (seen in October 2005) indicated an interaction between tillage and maize cultivar, but as these were only seen at the final sampling occasion it cannot be distinguished whether they were transient effects or would be perpetuated if the trial had continued. At the Narbons site there were data only from 2003 previously (Griffiths et al., 2005) when both CLPP and PLFA detected differences between maize and grass plots, with CLPP also detecting differences between maize cultivars. In this study (2004 and 2005), there were no differences between maize and grass detected by CLPP whereas PLFA did distinguish these treatments on all sampling occasions. This shows the benefit of determining microbial community structure by more than one method.

The methodology that we used to determine microbial community structure by fatty acid analysis changed from PLFA to ELFA. The reason for this was that the ELFA method is far quicker and easier than the PLFA method (Hinojosa et al., 2005), while the two methods are comparable in their discrimination (Drijber et al., 2000; Steger et al., 2003; Hinojosa et al., 2005). Our tests (data not shown), in which soils from the May 2004 samples were

analysed by both PLFA and ELFA, also showed that the two methods were comparable, hence the switch to ELFA in 2005. There was an increase in the total amount of fatty acid extracted by ELFA, compared to PLFA, but this has been observed in other studies (Hinojosa et al., 2005) and does not affect the interpretation of the proportional composition of the extracted fatty acids.

A pot experiment with different *Bt*- and non-*Bt*-cultivars to those used in the field at Narbons indicated that the insecticide used in the conventional crop management regime, Decis, had some effect on soil nematodes but no effect on protozoa (Griffiths et al., 2006). In the field study at Narbons, there were no differences in the abundance of nematodes under *Bt* or conventional crop management, but there was a reduction in protozoa under conventional crop management. The only difference between the two crop management regimes was the inclusion of the insecticide Decis in the conventional scheme. The effects on protozoa were, however, inconclusive in that they were transient (only seen in May 2004 and October 2005) and confounded by a maize cultivar interaction. In a strict test of the crop management regime, we used the same conventional cultivar (DKc5783) and observed the differences as mentioned. The experimental design also included another conventional cultivar, Paolis, with conventional crop management and a *Bt*-cultivar, DKc5784, with *Bt*-crop management. When these other cultivars were included, then the differences between conventional and *Bt* crop management were no longer significant ($P > 0.05$). This highlights the necessity of considering cultivar effects to put the magnitude of observed changes into context. The lack of consistent results between the pot and field experiments highlights a difficulty in extrapolating from the small scale to the large scale (see the paper by Birch et al., 2007, this volume). Interpretation is also complicated by the different soil types/field sites. Nematode community structure was affected by the HT-maize, although this maybe related to the different herbicide regime used as has been observed from the FSE for above-ground organisms (Haughton et al., 2003). Detailed glasshouse and laboratory experiments are under way to examine these effects in more detail. There was an effect of tillage on nematode community structure at both Foulum and Varois, although at Varois the effect was evident in 2005 but not in 2004 and there was also an effect of the *Bt*-trait at Varois but only in 2004 and only in the reduced-tillage plot. This might reflect a transitional effect of the change to reduced tillage having a transient interaction with the *Bt*-maize.

The interpretation of these data in a wider context, including information from other groups of organisms, has been facilitated by the application of a rule-based model (Bohanec et al., 2007, this volume).

Acknowledgements

ECOGEN is funded by contract QLK5-CT-2002-01666 from the European Commission. SCRI receives grant-in-aid from the Scottish Executive Environment and Rural Affairs Department.

References

Ahl, C., Joergensen, R.G., Kandeler, E., Meyer, B., Woehler, V., 1998. Microbial biomass and activity in silt and sand loams after long-term shallow tillage in central Germany. Soil Till. Res. 49, 93–104.

Andersen, M.N., Sausse, C., Lacroix, B., Caul, S., Messéan, A., 2007. Agricultural studies of GM maize and the field experimental infrastructure of ECOGEN. Pedobiologia 51, 175–184.

Birch, A.N.E., Griffiths, B.S., Caul, S., Thompson, J., Heckmann, L.H., Krogh, P.H., Cortet, J., 2007. The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: a review of the ECOGEN project. Pedobiologia 51, 251–260.

Bohanec, M., Cortet, J., Griffiths, B., Žnidaršič, M., Debeljak, M., Caul, S., Thompson, J., Krogh, P.H., 2007. A qualitative multi-attribute model for assessing the impact of cropping systems on soil quality. Pedobiologia 51, 239–250.

Bruinsma, M., Kowalchuk, G.A., van Veen, J.A., 2002. Effects of Genetically Modified Plants on Soil Ecosystems. Netherlands Institute of Ecology, Heteren, The Netherlands.

Bruinsma, M., Kowalchuk, G.A., van Veen, J.A., 2003. Effects of genetically modified plants on microbial communities and processes in soil. Biol. Fertil. Soils 37, 329–337.

Clark, B.W., Phillips, T.A., Coats, J.R., 2005. Environmental fate and effects of *Bacillus thuringiensis* (Bt) proteins from transgenic crops: a review. J. Agric. Food Chem. 53, 4643–4653.

Cortet, J., Andersen, M.N., Caul, S., Griffiths, B., Joffre, R., Lacroix, B., Sausse, C., Thompson, J., Krogh, P.H., 2005. Decomposition processes under *Bt* (*Bacillus thuringiensis*) maize: results of a multi-site experiment. Soil Biol. Biochem. 38, 195–199.

Darbyshire, J.F., Wheatley, R.E., Greaves, M.P., Inkson, R.H.E., 1974. A rapid method for estimating bacterial and protozoan populations in soil. Rev. Ecol. Biol. Sol. 11, 465–474.

Drijber, R.A., Doran, J.W., Pankhurst, A.M., Lyon, D.J., 2000. Changes in soil microbial community structure with tillage under long-term wheat fallow management. Soil Biol. Biochem. 32, 1419–1430.

Doran, J.W., Linn, D.M., 1994. Microbial ecology of conservation management systems. In: Hatfield, J.L., Stewart, B.A. (Eds.), Soil Biology: Effects on Soil Quality. Advances in Soil Science. Lewis Publishers, Boca Raton, FL, pp. 1–27.

Dunfield, K.E., Germida, J.J., 2004. Impact of genetically modified crops on soil- and plant-associated microbial communities. J. Environ. Qual. 33, 806–815.

Firbank, L.G., Heard, M.S., Woiwod, I.P., Hawes, C., Haughton, A.J., Champion, G.T., Scott, R.J., Hill, M.O., Dewar, A.M., Squire, G.R., May, M.J., Brooks, D.R., Bohan, D.A., Daniels, R.E., Osborne, J.L., Roy, D.B., Black, H.I.J., Rothery, P., Perry, J.N., 2003. An introduction to the farm-scale evaluations of genetically modified herbicide-tolerant crops. J. Appl. Ecol. 40, 2–16.

Frostegård, A., Tunlid, A., Bååth, E., 1991. Microbial biomass measured as total lipid phosphate in soils of different organic content. J. Microbiol. Methods 14, 151–163.

Garland, J.L., Mills, A.L., 1991. Classification and characterisation of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilisation. Appl. Environ. Microbiol. 57, 2351–2359.

Griffiths, B.S., Bonkowski, M., Dobson, G., Caul, S., 1999. Changes in soil microbial community structure in the presence of microbial-feeding nematodes and protozoa. Pedobiologia 43, 297–304.

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimgeour, C., Andersen, M.N., Cortet, J., Messéan, A., Sausse, C., Lacroix, B., Krogh, P.H., 2005. A comparison of soil microbial community structure, protozoa and nematodes in field plots of conventional and genetically modified maize expressing the *Bacillus thuringiensis* Cry1Ab toxin. Plant Soil 275, 135–146.

Griffiths, B.S., Caul, S., Thompson, J., Birch, A.N.E., Scrimeour, C., Cortet, J., Foggo, A., Hackett, C.A., Krogh, P.H., 2006. Soil microbial and faunal community responses to *Bt*-maize and insecticide in two soils. J. Environ. Qual. 35, 734–741.

Griffiths, B.S., Heckmann, L.H., Caul, S., Thompson, J., Scrimgeour, C., Krogh, P.H., 2007. Varietal effects of eight paired lines of transgenic *Bt* maize and near-isogenic non-*Bt* maize on soil microbial and nematode community structure. Plant Biotechnol. J. 5, 60–68.

Groot, A.T., Dicke, M., 2002. Insect-resistant transgenic plants in a multi-trophic context. Plant J. 31, 387–406.

Hackett, C.A., Griffiths, B.S., 1997. Statistical analysis of the time-course of Biolog substrate utilization. J. Microbiol. Methods 30, 63–69.

Haughton, A.J., Champion, G.T., Hawes, C., Heard, M.S., Brooks, D.R., Bohan, D.A., Clark, S.J., Dewar, A.M., Firbank, L.G., Osborne, J.L., Perry, J.N., Rothery, P., Roy, D.B., Scott, R.J., Woiwod, I.P., Birchall, C., Skellern, M.P., Walker, J.H., Baker, P., Browne, E.L., Dewar, A.J.G., Garner, B.H., Haylock, L.A., Horne, S.L., Mason, N.S., Sands, R.J.N., Walker, M.J., 2003. Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. II. Within-field epigeal and aerial arthropods. Philos. Trans. Roy. Soc. B 358, 1863–1877.

Hinojosa, M.B., Carreira, J.A., Garcìa-Ruíz, R., Dick, R.P., 2005. Microbial response to heavy metal-polluted soils: community analysis from phospholipids-linked fatty acids and ester-linked fatty acids extracts. J. Environ. Qual. 34, 1789–1800.

Holland, J.M., 2004. The environmental consequences of adopting conservation tillage in Europe: reviewing the evidence. Agric. Ecosyst. Environ. 103, 1–25.

Liu, B., Zeng, Q., Yan, F., Xu, H., Xu, C., 2005. Effects of transgenic plants on soil microorganisms. Plant Soil 271, 1–13.

Motavalli, P.P., Kremer, R.J., Fang, M., Means, N.E., 2004. Impact of genetically modified crops and their management on soil microbially mediated plant nutrient transformations. J. Environ. Qual. 33, 816–824.

Nielsen, P., Petersen, S.O., 2000. Ester-linked polar lipid fatty acid profiles of soil microbial communities: a comparison of extraction methods and evaluation of interference from humic acids. Soil Biol. Biochem. 32, 1241–1249.

O'Callaghan, M., Glare, T.R., Burgess, E.P.J., Malone, L.A., 2005. Effects of plants genetically modified for insect resistance on nontarget organisms. Annu. Rev. Entomol. 50, 271–292.

Petersen, S.O., Frohne, P.S., Kennedy, A.C., 2002. Dynamics of a soil microbial community under spring wheat. Soil Sci. Soc. Am. J. 66, 826–833.

Schutter, M.E., Dick, R.P., 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. Soil Sci. Soc. Am. J. 64, 1659–1668.

Spedding, T.A., Hamel, C., Mehuys, G.R., Madramootoo, C.A., 2004. Soil microbial dynamics in maize-growing soil under different tillage and residue management systems. Soil Biol. Biochem. 36, 499–512.

Steger, K.A., Jarvis, S., Smårs, S., Sundh, I., 2003. Comparison of signature lipid methods to determine microbial community structure in compost. J. Microbiol. Methods 55, 371–382.

Stotzky, G., 2004. Persistence and biological activity in the soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants. Plant Soil 266, 77–89.

The GenStat Committee, 2005. The Guide to GenStat® Release 8 – Part 2: Statistics. VSN International, Hemel Hempstead.

Wardle, D.A., 1995. Impacts of disturbance on detritus food webs in agro-ecosystems of contrasting tillage and weed management practices. Adv. Ecol. Res. 26, 105–185.

Winter, J.P., Voroney, R.P., Ainsworth, D.A., 1990. Soil microarthropods in long-term no-tillage and

conventional tillage corn production. Can. J. Soil Sci. 70, 641–653.
Whitehead, A.G., Hemming, J.R., 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Ann. Appl. Biol. 55, 25–38.
Yeates, G.W., Wardle, D.A., Watson, R.N., 1999. Responses of soil nematode populations, community structure and temporal variability to agricultural intensification over a seven-year period. Soil Biol. Biochem. 31, 1721–1733.