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REGULAR ARTICLE

Soil microbial and faunal responses to herbicide tolerant maize and herbicide in two soils

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Abstract A glasshouse experiment was set up to compare processes and organisms in two soils planted with genetically modified (GM) herbicide tolerant (HT) maize treated with appropriate herbicides. This was part of a wider project (ECOGEN) looking at the consequences of GM cropping systems on soil biology using a tiered approach at laboratory, glasshouse and field scales. Soil for the experiment was taken from field sites where the same maize cultivars were grown to allow comparison between results under glasshouse and field conditions. The maize cultivars T25 (GM HT glufosinate-ammonium tolerant), Orient (non HT near isogenic control for T25) and Monumental (a conventional, non HT variety) were grown in contrasting sandy loam and clay loam soils, half were sprayed with the appropriate herbicide as used in the field and soil samples were taken at the five-leaf and flowering plant growth stage. The main effects on all measured parameters were those of soil type and plant growth stage, with four categories of subsequent interaction: (1) there were no effects of herbicide on plant growth or soil microarthropods: (2) the maize cultivar (but not the GM HT trait) had effects on the decomposition of cotton strips and the nematode community; (3) herbicide application in general altered the community level physiological profile of the microbial community and reduced both soil basal respiration and the abundance of protozoa; and (4) the specific application of glufosinate-ammonium to T25 maize altered soil microbial community

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structure measured by ester linked fatty acids. The results from this glasshouse experiment support the findings from the field that there are effects of herbicide application on the soil microbial and meso-faunal community but that, compared to other standard agricultural practices, the differences are relatively small.

Keywords Decomposition · Genetically modified plants · Herbicide · Herbicide tolerant maize · Microbial community structure · Soil fauna

Introduction

The four major genetically modified (GM) crops grown are soybean, cotton, maize and oilseed rape (canola) with the two modified traits of herbicide tolerance and *Bt* insect resistance (James, 2006). There have been many studies on *Bt* plants and their effect on the soil environment due to root exudates and plant residues significantly increasing the concentration of *Bt* protein in soil with regard to non-target soil organisms (see for example a review by Stotzky 2004 and a special issue of Pedobiologia by Krogh and Griffiths 2007). There have been fewer studies to determine whether there is any effect on the soil community under a GM herbicide tolerant (HT) cropping regime and how it compares to the conventional cropping system. Motavalli et al. (2004) reviewed the direct and indirect effects of GM crops on soil nutrient transformations and concluded that although there were some negative effects associated with herbicides, the effects on plant growth were minimal. The adoption of HT crops has seen the progressive displacement of several pre-emergence herbicides such as atrazine and alachlor by post-emergence herbicides such as glyphosate or glufosinate. The post-emergence herbicides tend to require lower application and are less persistent chemicals in the environment (Scott and Pollak 2005) but with the increase in area of HT crops planted it is necessary to check for cumulative effects (Gómez-Barbero and Rodríguez-Cerezo 2006). One of the main pathways for pesticide degradation in soil systems is by microbial degradation (Mc-Ewen and Stephenson 1979), especially for glufosinate-ammonium (Bartsch and Tebbe 1989; Tebbe and Reber 1988) so it is important to maintain a healthy soil food web to maximise degradation rates (Behrendt et al. 1990). Previous studies have seen effects of several herbicides on the soil food web (Hendrix and Parmelee 1985; Malinda et al. 1982; Salminen et al. 1997; Yeates et al. 1993).

A practical consequence of the introduction of 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 (http://www.ecogen.dk) was initiated. Results from field experiments within ECOGEN revealed that both microbial and nematode community structure were affected significantly by an HT maize cropping system (Griffiths et al. 2007a). This might have been related to the different herbicide regime used, as was observed from the UK Farm Scale Evaluation for above-ground organisms (Haughton et al. 2003). The ECOGEN project adopted a tiered approach with parallel studies at field, glasshouse and laboratory levels (Birch et al. 2007). This paper reports on a glasshouse experiment to complement observations made at the ECOGEN field sites and toxicity tests on representative single species of soil fauna made in the laboratory.

Our objective was to examine the impact of maize varieties and herbicides used in conventional and GM cropping systems on the size and activity soil microbial and faunal populations, and to relate the findings to results observed in the field.

Materials and methods

Experimental design

Soil was collected from the ECOGEN field sites at Foulum, Denmark and Varois, France and transported to the Scottish Crop Research Institute. The soils, sites, general glasshouse experimentation and methods have been described in detail previously (Andersen et al. 2007; Griffiths et al. 2006). Soil from Foulum is a sandy loam (62.2% sand, 23.2% silt, 8.3% clay, pH $CaCl_2$ 5.6) containing 6.4% organic matter and that from Varois is a clay loam (20% sand, 41% silt, 30% clay, pH $CaCl_2$ 7.1) containing 4.8% organic matter. We used 22.7-cm diameter plastic pots filled with 6.0 kg mixed soil, sieved through a 6 mm diameter mesh, to which granular N–P–K fertilizer (22–4–14) (equivalent to 100 kg N ha^{-1}) was added. Pots were established in an environmentally controlled glasshouse with a 16 hour light period (20°C, with supplementary lighting activated at light levels <100 Wm^{-2}) and an 8 hour dark period (15°C) for 16 days before planting for biological activity to stabilise. The water content was maintained at a volumetric water content of 32% for the Foulum soil and 25% for the Varois soil, equivalent to a matric potential of −10 kPa, using an automated watering system together with regular manual additions to keep moisture fluctuations to a minimum. The soil water content and temperature was monitored using moisture probes and thermocouples attached to a data logger to record fluctuations throughout. Three days after potting an inoculum of diverse micro-arthropods extracted from local (i.e. from the Scottish Crop Research Institute) soil were added to each pot. Collecting and transporting soils to the UK for the experiment all but eliminated the soil micro-arthropod populations. To make the data from this glasshouse experiment as comparable to the field data from the ECOGEN project as possible, we added micro-arthropods from a local soil to ensure that we would have some information on this important faunal group. Micro-arthropods were extracted from 24 intact cores, 15 cm diameter and 10 cm deep, using Tullgren funnels as described below. Extracted micro-arthropods were combined and mixed in moist peat based compost which was divided equally and added to the surface of each pot, such that each pot received ca. 50 micro-arthropods.

The pots were arranged in a randomized block design that had three maize cultivars, T25 (GM HT glufosinate-ammonium tolerant), Orient (non HT near isogenic control for T25) and Monumental (a conventional, non HT variety), with and without herbicide application, in the two soil types (Foulum and Varois), with ten replications of each treatment giving a total of 120 pots. The ten replicate pots within each treatment were arranged in two blocks planted 1-week apart, one of six pots and one of four pots. All subsequent treatments, applications and samplings were time adjusted to maintain the temporal sequence between the blocks. A germinated maize seed was planted in the centre of each pot 4 cm below the surface. At the same time four autoclaved, pre-weighed standard cotton strips (10 cm×3 cm, soil burial shroud; Shirley Dyeing and Finishing Co., Hale, UK) were positioned just below the soil surface between the seed and pot to measure decomposition. Herbicide was applied to the soil at the equivalent of field application rates by spraying T25 with Basta (the equivalent of 2.5 L ha^{-1}, a.i. glufosinate-ammonium, Bayer Crop Science) 15 and 30 days after sowing, and the conventional varieties Orient and Monumental with Lido (1 L ha^{-1}, a.i. pyridate and terbuthylazin, Syngenta Crop Protection) 15 days after sowing followed by Calaris (0.5 L ha^{-1}, a.i. terbuthylazin and mesotrione, Syngenta Crop Protection) 30 days after sowing. The herbicides used were the same as applied at the ECOGEN field sites.

Sampling

Maize plants were harvested at the five leaf stage (week 6) or at flowering (week 12). At each harvest replicates of each treatment from each block were sampled. To maintain the temporal sequence between the blocks of pots, which had been planted one week apart, we sampled three replicates from the first block, followed 1 week later by two replicates from the second block, at each growth stage. The plant was cut from the pot, stems and leaves separated and dried at 50°C. The soil was tipped from the pots, sieved through a 6 mm diameter mesh and recovered roots and cotton strips were then washed and oven dried at 50°C. The remaining soil was stored overnight at 4°C prior to processing. Basal respiration was determined by incubating 10 g soil in a sealed 50 ml tube at 16°C for 24 h and measuring headspace CO_2 using an infrared gas analyzer (IRGA—ACD MGA 3000 Series). Nematodes were extracted from 200 g soil by decanting and sieving (Brown and Boag 1988), collected after 48 h, heat-killed for 2 min at 60°C and preserved in 4% formaldehyde. Total nematode numbers were counted then further processed through glycerol and mounted on a glass slide for identification. Protozoa (ciliates, flagellates and amoebae) were estimated by a most probable number technique (Darbyshire et al. 1974) involving serial dilution in a weak nutrient solution (Griffiths et al. 2006). Microarthropods were extracted from 700 g soil, over a five day period, using a Tullgren funnel apparatus (Burkard Manufacturing Co. Ltd., Rickmansworth, UK) and preserved in 70% ethanol.

Soil:saline suspension remaining from the protozoan measurement was used to determine the community-level physiological profile (CLPP. Garland and Mills 1991). The suspension was further diluted in sterile NMAS to give an absorbance of 0.4 at 595 nm and 150 µl inoculated into each well of a Biolog GN2 plate (Oxoid). The absorbance of each well at 595 nm was read initially and after incubation for 3, 4 and 5 days at 15°C.

For ester linked fatty acids (ELFA), frozen (−20°C) aliquots of soil were extracted with KOH in methanol, neutralized with acetic acid, fatty acid methyl esters extracted with hexane (Schutter and Dick 2000) and analysed by gas chromatography (Frostegård et al. 1991). The fatty acid nomenclature used below is that described by Ranneklev and Bååth (2003). Fatty acids i15:0, a15:0, i16:0, i17:0 and a17:0 were chosen to represent Gram positive bacterial markers (Joergensen and Potthoff 2005). The cyclopropane fatty acids 17cy and 19cy and the monoenoic and cyclopropane unsaturated ELFAs 16:19c and 16:17c were chosen to represent Gram negative bacterial markers (Johansen and Olsson 2005). Fatty acid 18:2ω6 was selected as a fungal marker (Frostegård and Bååth 1996). Fatty acid 10Me18:0 was chosen to represent actinomycetes (Shestak and Busse 2005). As such, the ELFA data give a qualitative insight into the microbial population and its structure. Total amount of ELFA was taken as an indicator of total microbial biomass.

All statistical analyses were performed using Genstat for Windows (version 9 The Genstat Committee 2005) or Canoco for Windows 4.5 (Ter Braak and Smilauer 2002). The time-course profiles of the CLPP data were analysed from the area under the colour development profile (Hackett and Griffiths 1997). CLPP area under the curve and ELFA data were analysed by principal component (PC) analysis, with the resulting PCs and other data being analysed by ANOVA, correlations or covariance matrix using soil type, plant growth stage, maize variety and herbicide application as treatment factors, and the time of planting as a blocking factor. Microarthropods were analysed by a Correspondance Analysis (CA), correlation matrix using soil type, plant growth stage, maize variety and herbicide application as treatment factors. Some data were transformed (amounts by natural logarithm or square root and percentages by an angular transformation) to stabilise variances and, in those cases, the detransformed mean and the transformed standard error of the difference of the mean (SED) are presented.

Results

Environmental conditions Soil temperature was the same regardless of treatment with a mean of 18°C, but varying between 13–26°C. Volumetric soil water content varied between watering intervals, from 10–40% as minimum and maximum during the experiment.

Plant growth Data were log transformed for analysis. There was a significantly ($P<0.001$) greater plant biomass in soil from Varois (overall mean 16.5 g) than from Foulum (12.0 g, SED 0.0323) and a maize variety × growth stage interaction ($P<0.05$). At the five-leaf stage, Monumental (4.1 g), Orient (5.7 g) and T25 (4.9 g) all differed significantly, while at the flowering stage Orient (42.8 g) and T25 (46.9 g) were significantly heavier than Monumental (35.0 g) but not significantly different from each other. Root weight was significantly ($P<0.05$) greater in soil from Varois (0.78 g) than Foulum (0.65 g, SED 0.072) at the five-leaf stage, but there were no significant differences at flowering (4.7 and 4.8 g respectively). There was a significant maize variety × growth stage interaction ($P<0.001$), with Orient having a grater root mass (0.8 g) than T25 and Monumental (0.7 g each) at the five-leaf stage but a smaller root mass (3.9 g) than T25 (5.3 g) and Monumental (5.1 g, SED 0.087) at flowering.

Cotton strip decomposition There were significant effects of maize variety ($P<0.001$) and a soil × growth stage interaction ($P<0.05$). There was greater decomposition under T25 and Monumental (66% and 60% weight loss respectively) than under Orient (51%, SED 0.035). At the five-leaf stage, decomposition was the same in soil from Foulum and Varois (48% and 44% weight loss respectively), while at flowering there was significantly greater decomposition in soil from Varois (77% weight loss) than from Foulum (66% weight loss, SED 0.039).

Basal respiration There was a significant soil× growth stage interaction ($P<0.001$), as there was no significant difference in respiration rate between soil from Varois and Foulum at the five-leaf stage (1.37 and 1.45 μg CO_2 g^{-1} h^{-1} respectively) but at flowering both soils respired significantly faster than at the five-leaf stage and soil from Varois respired significantly faster than soil from Foulum (2.13 and 1.72 CO_2 g^{-1} h^{-1} respectively, SED 0.071). There was also a significant maize variety×soil interaction ($P<0.01$), there being no significant difference in the respiration rate in soil from Varois under Monumental, Orient or T25 (1.75, 1.71 and 1.80 CO_2 g^{-1} h^{-1} respectively) but in soil from Foulum respiration rate under Monumental was significantly slower than under Orient or T25 (1.39, 1.74 and 1.63 CO_2 g^{-1} h^{-1} respectively, SED 0.086). The respiration rate under Monumental in soil from Varois was significantly faster than in soil from Foulum, but there were no such differences under the other maize varieties. There was a significant ($P<0.05$) effect of herbicide application, with respiration in soils treated with herbicide) being lower than control soils without herbicide (1.61 and 1.72 μg CO_2 g^{-1} h^{-1} respectively, SED 0.05).

Nematodes Abundance data were log transformed and community composition (%) data were given an angular transformation for analysis. There was a significant ($P<0.05$) soil×maize variety×growth stage interaction (Fig. 1). There were more nematodes in soil from Foulum than Varois under Monumental at the five-leaf stage and under all maize varieties at flowering. At the five-leaf stage soil from Foulum under Monumental and T25 contained fewer nematodes than under Orient, while soil from Varois under Orient and T25 contained fewer than under Monumental. At flowering there were no significant differences in nematode abundance between the maize varieties regardless of soil type. Nematode abundance significantly declined between flowering and the five-leaf stage under Orient and T25 but not Monumental. There was also a significant soil×maize variety×herbicide interaction ($P<0.05$, Fig. 1). Herbicide effects were only significant in soil from Varois, where nematode abundance was increased

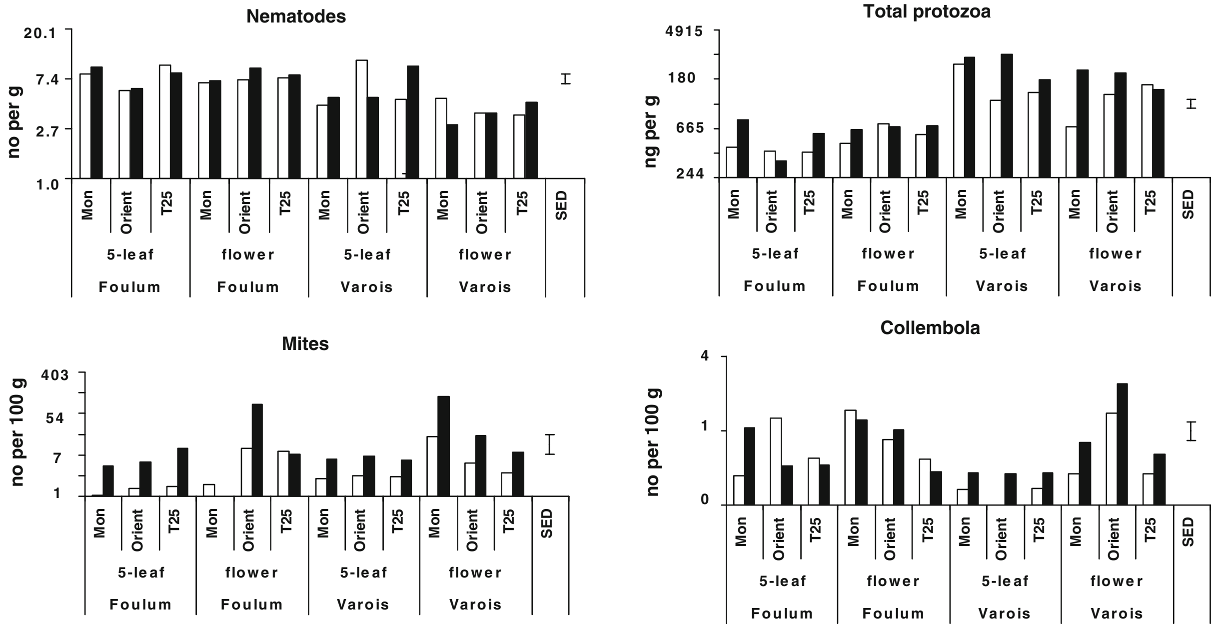

Fig. 1 Abundances of: nematodes; total protozoa and mites (all L_n transformed for analysis); and Collembola (square root transformed for analysis) in soils treated (open symbols) or not treated with herbicide (*solid symbols*). Maize varieties Monumental (*Mon*) and Orient were treated with Lido and Calaris, while the GM T25 was treated with Basta. Soils from Foulum and Varois were sampled at the five-leaf and flowering stages of growth. Bars represent the standard error of the difference of the mean (SED) for: nematodes−soil×maize variety×growth stage interaction; total protozoa−soil×growth stage interaction; mites−soil×maize variety×herbicide interaction; Collembola−soil×maize variety×growth stage interaction. $n=5$

under T25 and decreased under Orient. Analysis of the nematode community at the five-leaf stage revealed effects of soil type and some effects due to the maize cultivar, but no significant ($P>0.05$) herbicide effects or interactions. Soil from Foulum contained relatively more: *Acrobeloides*; *Aporcelaimellus*; *Paratylechus*; *Pratylenchus*; *Teratocephalus*; omnivores and plant feeders than soil from Varois, while soil from Varois contained relatively more: Aphelenchidae; *Eucephalobus*; *Plectus*; Tylenchidae and fungal feeders than soil from Foulum (Table 1).

Protozoa Abundance data were log transformed for analysis. Effects on total protozoan biomass (Fig. 1) were largely driven by changes in amoebae, rather than flagellates or ciliates. There was a significant soil × growth stage interaction ($P<0.05$) with a greater protozoan biomass in soil from Varois than Foulum but a greater difference between the two soils at the five-leaf stage than at flowering. There was a significant ($P<0.05$) overall effect of herbicide, with protozoan biomass reduced in soils treated with herbicide (overall mean protozoan biomass without herbicide was 1143 ng g^{-1} and with herbicide this was reduced to 822 ng g^{-1}, SED of the transformed data was 0.193).

Microarthropods Mite abundance data were log transformed for analysis. There was a herbicide × maize variety × soil interaction ($P<0.05$), with fewer mites in herbicide treated than untreated soil, but only with soil from Varois and only with Monumental. Abundance also significantly ($P<0.05$) increased with plant growth stage (Fig. 1). Collembolan abundance data were square root transformed for analysis and there was a significant maize variety × soil × growth stage interaction ($P<0.05$). In soil from Foulum there were more Collembola under Monumental than T25, while in soil from Varois there were more under Orient than the other varieties and this was only significant at the flowering stage (Fig. 1). The Collembola in the inoculum were identified as: *Protaphorura armata*, *Parisotoma notabilis*, *Mesaphorura* sp., *Folsomides parvalus*, *Isotoma* sp., *Bourletiella hortensis* and *Sminthurinus elegans*. Although equal populations were added to each pot, the final populations in the two soil types were significantly ($P<0.05$) affected by soil type and plant growth stage. The first axis of the Correspondance Analysis (29% of variation) discriminated on soil type, with soil from Foulum being associated with *B. hortensis, S. elegans and P. armata* and soil from Varois being associated with *Mesaphorura sp.*, *P. notabilis* and *F. parvulus*. The second axis (21% of variation) discriminated on plant growth stage when flowering was associated with *P. notabilis*. The other factors were less important in the variation and not clearly discriminated by the analysis.

Table 1 Trophic structure (% composition of bacterial- (BF), fungal- (FF), plant-feeding (PF), omnivorous and predatory (OM) nematodes) of the nematode communities in soil at the five-leaf stage of maize growth

Soil	Maize	Herbicide	%BF	%FF	%OM	%PF
Foulum	Monumental	Lido/Calaris	53	17	12	17
		None	55	18	12	12
	Orient	Lido/Calaris	55	24	7	8
		None	51	21	14	13
	T25	Basta	52	18	11	18
		None	47	22	20	10
Varois	Monumental	Lido/Calaris	52	45	0	1
		None	53	43	0	2
	Orient	Lido/Calaris	54	46	0	0
		None	51	47	1	0
	T25	Basta	57	32	4	2
		None	54	42	1	1
SED[a]	Soil		0.032	0.032	0.027	0.027
SED[a]	Maize		0.039	0.039	0.033	0.033

[a] Standard Error of the Difference of the mean for angular transformed data, means in table are de-transformed.

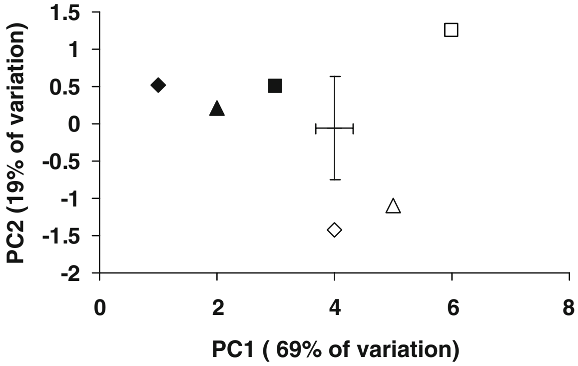

Fig. 2 Mean principal component scores of the ELFA profiles of soils treated (*open symbols*) or not treated with herbicide (*solid symbols*). Maize varieties Monumental (*diamonds*) and Orient (*triangles*) were treated with Lido and Calaris, while the GM T25 (*squares*) was treated with Basta. Scores have been meaned over soils and plant growth stage. *Bars* represent the least significant difference, $P<0.05$, $n=20$

CLPP The first four principal components of the CLPP explained 24%, 7%, 5% and 4% of the variation respectively. Analysis of variance of there components showed significant (P<0.001) effects of both soil type and growth stage in PC1 and PC2. PC3 (5% of the variation) showed a significant ($P<0.05$) growth stage×soil type×herbicide interaction, indicating a herbicide effect on the CLPP at the five-leaf stage in soil from Foulum. PC4 gave significant interactions between soil×maize variety ($P<0.05$) and growth stage×soil ($P<0.001$) and a herbicide effect ($P<0.05$). By analysing each soil type and growth stage combination separately, so as to remove their overwhelming effects when comparing between soil type and growth stage, significant ($P<0.05$) effects of herbicide application were evident in soil from Foulum at the five-leaf and flowering stages and in soil from Varois at the flowering stage. There were no significant ($P>0.05$) interactions with maize variety so that the type of herbicide applied had no effect on the CLPP.

ELFA While there were highly significant effects of soil type and growth stage ($P<0.001$) on the ELFA pattern, PC1 (69% of variation) also had significant effects due to the maize variety ($P<0.05$) and a maize variety×herbicide interaction ($P<0.01$). Similarly, PC2 (19% of variation) had significant effects of maize variety ($P<0.05$), herbicide application ($P<0.05$) and a maize variety×soil type×herbicide interaction ($P<0.05$). Here it was the maize varieties Orient and Monumental that differed from T25, only when herbicide was applied and only in soil from Varois (Fig. 2). When the concentrations of biomarker fatty acids were compared there was a relative increase in the Gram −ve bacteria and decrease in Gram + ve bacteria and actinomycetes in soil from Varois treated with Basta (Fig. 3). The total amount of ELFA extracted from the soils was significantly ($P<0.001$) greater in soil from Foulum than from Varois (119.5 and 95.8 nmol g^{-1} respectively) and from soils at the flowering then the five-leaf growth stage (115.4 and 99.9 nmol g^{-1} respectively).

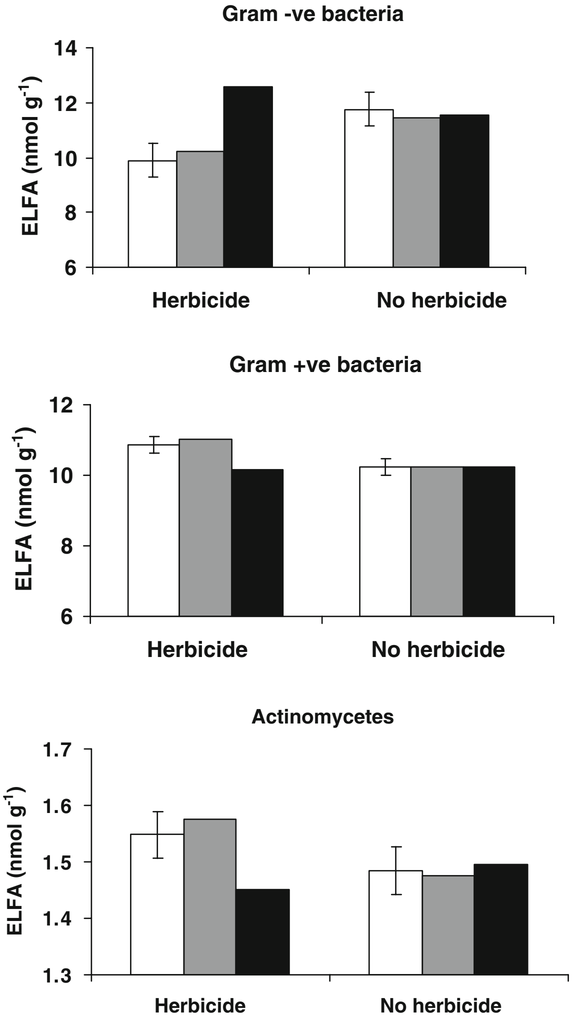

Fig. 3 Concentration of biomarker ELFAs in soil from Varois planted with one of three maize varieties (Monumental, Orient, or T25: *white*, *grey*, *black* respectively) and treated with herbicide or not. Scores have been meaned over plant growth stage. *Bars* represent the least significant difference ($P<0.05$), $n=10$

Discussion

The consensus from our range of population and process determinations in this pot experiment was that the largest effects were due to soil type and plant growth stage, with less pronounced effects arising from herbicide application and maize variety differences (summarised in Table 2). We also found the same order of responses in a glasshouse experiment with *Bt* maize (i.e. soil type and plant growth stage had much larger effects than maize variety or insecticide application, Griffiths et al. 2006). The findings generally agree with the main body of results arising from studies of a range of herbicides on soil organisms. A review by Wainwright (1978) concluded that herbicides have little deleterious influence on soil processes when applied at field rates. Although under certain conditions there have been: reductions in bacteria and fungi (Ahmed and Malloch 1995, with Basta; Lin and Brookes 1999, with Dinoseb); changes in microbial community structure without concomitant changes in microbial function (Seghers et al. 2003, 2005, with nicosulfuron, dimethenamide, atrazine and metolachlor; de Lipthay et al. 2004, with mecoprop, dichlorprop, 4,6-dinitroorthocresol (DNOC), bentazone, isoproturon and 2,4-dichlorobenzamide; Gonod et al. 2006, with 2,4-D; Rouard et al. 1996, with DNOC; Fließbach and Mäder 2004, with Basta and Dinoseb); altered microbial function (Baruah and Mishra 1986, with 2,4-D, butachlor and oxyfluoron); as well as no detectable effects (Ratcliff et al. 2006, with glyphosate). Effects on soil animals indicate that herbicides can influence soil community structure and function, both directly through effects on soil organisms and indirectly through effects on supporting plant communities (Hendrix and Parmelee 1985, with atrazine, paraquat and glyphosate; Yeates et al. 1993, with atrazine and rimsulfuron; Malinda et al. 1982, with Hoegrass and Buctril; Salminen et al. 1997, with terbuthylazine). The consequences of growing GM-HT crops for soil organisms have more recently been studied in the field. In studies of Basta-tolerant maize, such as used in this study, neither the GM maize nor the herbicide affected rhizosphere microbial community structure (Schmalenberger and Tebbe 2002), although Griffiths et al. (2007a) noted transient effects on microbial and nematode community structure. Similarly, shifts in microbial community structure

Table 2 Summary of the significant effects and interactions of soil type, growth stage, herbicide application and maize variety on plant growth and soil microbial parameters

Parameter	Single Effects				Interactions							
	Soil (S)	Growth Stage (G)	Herbicide (H)	Maize (M)	Sx G	Gx H	Gx M	Sx M	Hx M	Sx H	SxH xM	SxG xM
Plant weight	***	***		***	***		***					
Respiration	***	***	*	*	***			**				
Decomposition	***	***		***	**							
Total ELFA	***	***										
ELFA	***	***	*	*	***		*		**			
CLPP	***	***	*	*	***			*				
Protozoa	***		*		*							
Amoebae	***		*							*		
Ciliates	***											
Flagellates	**			**	*							
Nematodes	***	***			**						**	*
Collembola	*	***		*	*							*
Mites	*	*							*		*	

Total ELFA is the total amount extracted (equivalent to microbial biomass), while ELFA and CLPP are the principal component scores from analysis, see text for details.

***$P<0.001$

**$P<0.01$

*$P<0.05$

due to glyphosate tolerant wheat and oil seed rape were minor and inconsistent (Lupwayi et al. 2007).

An objective of the ECOGEN project was to undertake a tiered approach to risk assessment (Angle 1994) by including laboratory, glasshouse and field experiments (Birch et al. 2007). Results from the field at the cropping system level include indirect effects, for example the amount and duration of weeds in the plots resulting from the herbicide regime, which can largely be excluded from the glasshouse experiment. While in the laboratory the direct effects of the herbicides on selected model organisms can be tested. There were reduced abundances of nematodes (*Caenorhabditis elegans*) and ciliate protozoa (*Colpoda steinii*) in soil from Foulum treated with realistic concentrations of Lido, while for Basta there were no significant effects on the nematode but a reduced ciliate abundance (S. Caul, personal communication). Thus, some direct effect of the herbicides on soil organisms might be expected but the results from the higher level tiers shows that these were largely offset by the longer timescales, larger soil volumes and interactions with a complex soil community.

Methodologically there was a problem with the cotton strip decomposition assay. Normally the method measures the reduction in tensile strength as the index of decomposition (Harrison et al. 1988) but as the strips were too degraded at even the five-leaf stage to allow such a measurement a weight loss seemed to be the most practical alternative. The measurements of decomposition were, therefore, probably less precise than they might have been. Nevertheless, results of the statistical analyses showed that, even when the dominating effects of soil and plant growth stage were removed, the effects of maize variety and herbicide application were inconsistent. In soils with the non-GM isogenic variety Orient, there was less decomposition of the cotton strips than with either the GM T25 or the non-GM Monumental, but no effects of herbicide. Such varietal differences are to be expected (Griffiths et al. 2007b) and in some ways act as a yardstick by which to estimate the magnitude of changes due to herbicide or the GM-variety+herbicide combination. Respiration, however, was greater with both Orient and T25 than Monumental, which matched the differences in plant growth, but respiration was also significantly reduced by herbicide (both Basta and Lido/Calaris). Protozoa and microarthropods similarly showed a significant reduction after herbicide (both Basta and Lido/Calaris) but only in soil from Varois. The measures of microbial community structure, ELFA and CLPP, were differently affected by maize variety and herbicide. CLPP was affected by the herbicide treatment (both Basta and Lido/Calaris) in interactions with soil and plant growth stage, whereas ELFA revealed a distinct maize variety×herbicide interaction. In soil from Varois at the flowering stage the T25+Basta combination had a significantly different microbial community structure from the other treatments. Indicating that the effect was a result of the different effects of the herbicides Lido/Calaris compared to Basta in soil from Varois. This was the only indication of a specific herbicide (i.e. Basta giving a different response to Lido/Calaris) interaction. The significant effects were noted at the five-leaf stage rather than later on at the flowering stage, which may indicate an initial effect of the herbicide application from which the soil microbial community recovers over time. The active ingredients of the herbicides have a contact rather than a systemic mode of action, apart from mesotrione (a component of Calaris) which can also be absorbed by the roots and translocated. It is likely that the herbicide effect is due to differences in chemical composition rather than mode of action. A difference in the results of CLPP and ELFA has been noted before, in field and glasshouse experiments (Griffiths et al. 2005, 2006, 2007a), which are clearly measuring different aspects of the microbial community.

The results from this glasshouse experiment support the findings from the field (Cortet et al. 2007; Griffiths et al. 2007a) that there are effects of herbicide application on the soil microbial and faunal community but that, compared to other standard agricultural practices, the differences are relatively small. None of the effects seen in the T25+Basta combination could be attributed to the genetic modification of the maize variety. Impacts on other soil fauna may be greater. In field plots at Foulum growing HT maize, for example, there was a significant reduction in earthworm abundance and biomass that may have been caused by the herbicide (Krogh et al. 2007).

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