

Monitoring carabid indicators could reveal environmental impacts of genetically modified maize

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- Abstract**
- 1 Post market monitoring of GM crops is mandatory in EU states where they are grown commercially. Carabid beetles are very sensitive to environmental changes and may be used as indicators to monitor impacts of transgenic crops. This study aimed to assess which, where and when carabids should be sampled for improved postmarket monitoring.
 - 2 Carabids were pitfall-trapped in maize fields, field margins, alfalfa and semi-natural vegetation across three regions of NE Spain, during 2 years, and three sampling dates.
 - 3 Overall, 9193 carabids of 42 species were identified, aggregated into trophic groups and used for calculating community measures. The best indicator was *Pseudoophonus rufipes* (De Geer), satisfying criteria of abundance, relevance, sensitivity, ease of sampling, and sufficient statistical power. The carnivore group should also be monitored as an indicator of biodiversity and invertebrate biological control.
 - 4 The best sampling location was the field margin where carabids are exposed to GM maize and are abundant enough to require smaller sample sizes to detect population changes.
 - 5 Finally, sampling should concentrate around maize pollen-shed when carabid abundance is highest.
 - 6 This study provides baseline data and shows that carabids can cost effectively improve detection capacity of postmarket monitoring.

Keywords Biotechnology, Bt maize, corn, genetic engineering, ground beetle, herbicide tolerant maize, pest management, risk assessment.

Introduction

Genetically modified (GM) maize is cultivated on a commercial scale in Spain, where *Bacillus thuringiensis* (*Bt*) maize (event MON810) occupied almost 132 000 ha in 2014 (James, 2014). Although this is the only GM crop produced in the European Union (EU), maize varieties containing other insect resistance (*Bt*) and herbicide tolerance (GMHT) traits are in the authorization process. As a precautionary measure, EU legislation (Annex VII of Directive 2001/18/EC and Council Decision 2002/811/EC) requires post-market environmental monitoring (PMEM) after placement on the market of any GM crop. As part of the PMEM, general surveillance (GS) aims to detect cumulative, delayed or unexpected adverse effects of GM crops on human health or the environment (EFSA, 2006). This

long-term monitoring is compulsory even if the environmental risk assessment identified no potential risks. Currently, GS guidelines (EFSA Panel on Genetically Modified Organisms, 2011, 2014) recommend the use of three approaches: (i) monitoring of the crop and its cultivation sites through a farm questionnaire; (ii) use of data collected by existing environmental surveillance networks; and (iii) analysis of data from the scientific literature. The implementation of a specific plan to monitor for adverse effects of GM crops is not contemplated in EU legislation despite the fact that farm questionnaires could not reveal changes in overall biodiversity, many agricultural areas lack appropriate environmental surveillance networks and the scientific literature may not focus on relevant indicators. If GS is to serve its purpose, it should include indicators capable of revealing adverse effects of GM crops on ecosystem biodiversity and functions.

Carabids (Coleoptera: Carabidae) are widely used as indicators because they respond to environmental change (Rainio & Niemelä, 2003) and they are easy to collect by pitfall-trapping (Kotze *et al.*, 2011). They are particularly suitable for monitoring

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environmental effects of GM maize because they feed and breed within crop fields (Kromp, 1999), being more exposed to GM crops than other indicators proposed for this purpose, such as butterflies (Perry *et al.*, 2010). Moreover, because of their mobility, within-field impacts could carry over to neighbouring habitats. They are taxonomically diverse and provide important ecosystem services via the control of invertebrate pests (Kromp, 1999) and weed seeds (Honek *et al.*, 2006; Bohan *et al.*, 2011). Their wide distribution and abundance in agricultural systems (Holland & Luff, 2000) allow for statistical analysis (Legendre & Legendre, 1998; Comas *et al.*, 2015). Finally, their taxonomy and ecology are sufficiently well known to enable their identification with relative ease and population changes to be interpreted (Rainio & Niemelä, 2003; Kotze *et al.*, 2011).

Carabids respond to agricultural management (Holland & Luff, 2000; Döring & Kromp, 2003; Aviron *et al.*, 2005; Legrand *et al.*, 2011) and so they can be expected to respond to GM cropping through several mechanisms. Although GS is not hypothesis driven (EFSA Panel on Genetically Modified Organisms, 2011), the probable exposure pathways to GMHT and *Bt* maize should be evaluated to select appropriate indicators.

The main mechanism by which cultivation of GMHT maize may affect carabids is via changes in herbicide type, as well as the timing and number of applications, compared with cultivation of conventional maize (Brooks *et al.*, 2005; Heard *et al.*, 2006). These changes could affect carabids directly through herbicide toxicity, which is very rarely reported in the literature (Brooks *et al.*, 2005; Michalková & Pekár, 2009). Alternatively, the impact may be indirect, as a result of shifts in weed density, phenology, distribution and composition (Heard *et al.*, 2006; Smith *et al.*, 2008; Albajes *et al.*, 2009), because weeds provide food resources and control the microclimate for carabids (Holland & Luff, 2000). Such changes in weed populations and assemblages affect carabids differently according to their feeding preferences (Purtauf *et al.*, 2005; Vanbergen *et al.*, 2010). For example, in the U.K. Farm Scale Evaluation of GM crops (Perry *et al.*, 2003), higher weed density in GMHT maize increased the number of seed-feeding carabids (Brooks *et al.*, 2003; Heard *et al.*, 2006). This effect on seed-feeding carabids was maintained when different herbicide regimes were assessed (Brooks *et al.*, 2005). In a 4-year study, Albajes *et al.* (2011) indicated that moderate changes in herbicide regimes did not affect predator densities, despite lower weed densities in GMHT maize, and only a dramatic weed alteration affected the abundance and composition of predatory fauna. Indeed, in conventional maize, broad spectrum herbicides can only be applied prior to crop emergence, when the early removal of all plant cover may hinder colonization by carabid predators (Brooks *et al.*, 2005). This can result in a lower biological control of insect pests regarding GMHT maize, where broad spectrum herbicides may be applied later in the season (Bigler & Albajes, 2011).

Bt crops produce taxon-specific insecticidal Cry proteins, toxic by ingestion (Gill *et al.*, 1992). Thus, organisms taxonomically close to the target insects (in maize Lepidoptera and Coleoptera) are potentially more susceptible. Exposure to *Bt* toxins is greatest for species feeding on GM maize but carabids may also be exposed by accidental ingestion of pollen (Stanley-Horn *et al.*, 2001; Lepping, 2009), exposure to root exudates (Icoz & Stotzky, 2008), consumption of contaminated prey (Obrist

et al., 2006) or lower numbers and quality of prey (Meissle *et al.*, 2005; Naranjo, 2009). So far, however, for Lepidoptera-resistant maize no detectable effects on carabids have been reported in field studies (De La Poza *et al.*, 2005; Lopez *et al.*, 2005; Kocourek *et al.*, 2013; Comas *et al.*, 2014), although Meissle *et al.* (2005) detected adverse effects on carabids fed with larvae that had ingested *Bt* toxins. The situation was similar for Coleoptera-resistant maize. Most studies reported no effects (Lepping, 2009; Leslie *et al.*, 2010), although one study found a reduction in overall carabid numbers for which the mechanism was unknown (Stephens *et al.*, 2012). However, the lack of consistent adverse environmental effects of *Bt* maize could simply indicate that the timescale of these studies was insufficient to detect subtle population effects, which may only be revealed after decades of generalized cultivation.

In previous field trials carried out in northeast Spain (Eizaguirre *et al.*, 2006; Albajes *et al.*, 2009), the carabid group was sufficiently abundant to detect population changes in *Bt* maize at low sample sizes (Albajes *et al.*, 2013; Comas *et al.*, 2013). Nevertheless, using overall carabid number for monitoring does not reveal how GM cropping may be affecting carabid diversity or the ecosystem functions that they carry out.

The present study outlines an improved environmental monitoring plan that would make GS more likely to detect any potential adverse effects of *Bt* and GMHT maize cultivation on ecosystem diversity and functions. The specific aims were: (i) to assess abundance and frequency of carabid species present in the maize agroecosystem across regions and years, providing baseline information; (ii) to determine the most suitable carabid indicators for standardized monitoring based on distribution, statistical power, sample size, and capacity for reflecting ecosystem diversity and functions; and, finally; (iii) to determine the most suitable sampling sites and dates among those tested in the present study.

Materials and methods

Study regions

Carabids were sampled in three different maize cropping regions in northeast Spain to account for variability as a result of differences in landscape, cultural practices and agroclimatic conditions. The Bujaraloz study region (41°29'50"N 0°9'13"E) is located in the Monegros badlands at 350 m a.s.l.; Almacelles (41°43'57"N 0°26'25"E) is 250 m a.s.l. in the Lleida agricultural plains; and, finally, La Seu (42°21'32"N 1°27'43"E) is 691 m a.s.l. in the Pyrenees. Climate and cultivation practices are similar in Bujaraloz and Almacelles where maize is cultivated for grain; here, intensive soil cultivation, applications of pre-emergence herbicides and deployment of *Bt* maize (event MON810) for cornborer control are common practice. Almacelles landscape is a mosaic of arable crops and orchards; semi-natural vegetation is confined to hills, waterways and paths. In Bujaraloz, fields are larger and the landscape is composed of arable crops and large uncultivated patches. Finally, in La Seu, maize is cropped for silage as part of a yearly crop rotation; agricultural practices include no-till and pre-emergence herbicide applications. *Bt* maize is not used because there is no cornborer pressure; landscape is a mosaic of forage crops, pastures and

forest. Average maize field sizes were 8.4 ± 1.45 ha in Bujaraloz, 5.4 ± 0.9 ha in Almacelles and 3 ± 0.7 ha in La Seu.

Carabid sampling and identification

Ten conventionally managed maize fields (five in 2011 and five more in 2012) were sampled in each study region (Bujaraloz, Almacelles and La Seu). Three neighbouring habitats were sampled when present (29 field margins, 27 alfalfa fields and 19 areas of semi-natural vegetation). Each field and its neighbouring habitats were sampled three times: in July, during maize vegetative stages (V10–V14); in August, around pollen-shed (VT–R1); and, in September, at ripening of the grain (R4–R6). A line of three pitfall traps, 10 m spaced apart, was placed in the sampling site 15–20 m from the edge, where possible. Only three traps were used because the present study aimed to outline a feasible monitoring plan and this number of traps had previously been used successfully by our research group (De La Poza *et al.*, 2005). Traps consisted of a buried plastic sheath in which a glass jar (diameter 9 cm, depth 17 cm) was placed flush with the soil surface and filled with 250 mL of a 20% solution of propylene glycol and water, covered with a polystyrene tray placed 2 cm above the ground to prevent flooding and reduce vertebrate bycatch. Traps were left active for 1 week each time and covered when not in use.

Carabids were identified in accordance with previous studies (mainly Trautner & Geigenmüller, 1987; Luff, 2007), with nomenclature *sensu* Serrano (2003). After expert revision by E. Vives, Natural History Museum of Barcelona, Spain, voucher specimens were stored at Lleida University. Trophic groups, indicating ecosystem function, were assigned according to previous studies (Laroche, 1990; Purtauf *et al.*, 2005; Ameixa & Kindlmann, 2008; Vanbergen *et al.*, 2010). Criteria for assigning trophic groups were reports of adult beetles feeding solely on animal material (carnivores), solely on plant material (phytophages) or on both (omnivores).

In pitfall trapping, the abundances of a species in a trap are influenced not only by the abundance of the species in the environment, but also by its activity (Honek, 1988). Therefore, the abundance of the carabid species collected by this method is only a proxy of the true composition and size of carabid populations. Nevertheless, for simplicity, the term ‘abundance’ is used throughout the present study to refer to the number of carabids collected in pitfall traps.

Carabid community measures

Species richness and Shannon’s diversity index were calculated for each plot, aiming to determine the potential value of carabids as biodiversity indicators. Because the observed species richness is a function of sample size (Gotelli & Colwell, 2001), Chao 1 index was used to estimate true species richness (Chao, 1987), which is the predicted value considering number of unrecorded but present species (Colwell, 2013). Similarity of carabid assemblages between habitats was calculated using the estimated abundance-based Chao-Jaccard similarity index (Chao *et al.*, 2005). This was carried out to assess the similarity of maize carabid assemblages compared with assemblages in

other habitats. In this way, information can be obtained on the suitability of monitoring the impacts of GM maize in habitats other than maize. These calculations were conducted using ESTIMATES, version 9.1 (Colwell, 2013).

Linear relationships for abundance between carabid species and groups compared with community measures were tested by Pearson’s correlation coefficient, aiming to evaluate their potential as indicators of carabid diversity (Duelli & Obrist, 2003).

Frequency, proportion of fields where a species was present, and dominance, with species together constituting 95% of relative abundance (Luff, 2002), were calculated to identify the most common and abundant species. These species are potentially the most valuable for use as standardized indicators of GM maize impacts.

Habitat, region and year as sources of baseline variability

For each habitat and sampling site, mean values of community measures and mean abundance of carabid species and trophic groups were calculated. The effects of the main factors habitat, region and year and their interactions on these indicators were assessed to obtain information on the sources of variability of baseline carabid data. Data were analyzed with a generalized linear model (GLM), using a Poisson distribution and log-link function (Gaussian distribution and identity-link function for community measures). Because the interactions between the main factors were significant for most carabids, further analyses were carried out in two steps.

In the first step, variation partitioning was performed to determine how much of the variation of the final GLM model was explained by the pure effect of each factor (habitat, region and year) and which proportion was attributable to their shared effect (Whittaker, 1984; Legendre & Legendre, 1998). Variation partitioning was carried out with the VarPart function in the R package ModEva (Barbosa *et al.*, 2014) and calculated by using the squared value of Spearman’s correlation coefficient between the values of the final model and a model based only on the pure factor. Negative values represent opposing effects. The goodness of fit statistic, analogous to r^2 , representing the proportion of deviance explained by the GLM model, was approximated by $D^2 = 1 - [\text{residual deviance}/\text{null deviance}]$ (Yee & Mitchell, 1991).

In the second step, regional and year-to-year variation was examined within each habitat (30 maize fields, 29 field margins and 27 alfalfa plots); the semi-natural habitat was not analyzed as a result of low carabid abundance. Significant differences were determined by a chi-squared test ($\alpha = 0.05$) and explored using Tukey’s honestly significant difference. Analyses were carried out with R (R Core Team, 2013) using the MASS package (Venables & Ripley, 2002).

Long-term population fluctuation of common carabids

Historic data from 2005 to 2012 obtained by our research group from field studies on nontarget effects of GM maize carried out in the Lleida plains (Comas *et al.*, 2014), where Almacelles is located, were used to determine the dominant species of the

Table 1 Carabid species collected from pitfall traps in maize fields (*n*) in three regions of northeast Spain

Species in maize	TG	Bujaraloz		Almacelles		La Seu	
		(<i>n</i> = 10) AD	<i>F</i>	(<i>n</i> = 10) AD	<i>F</i>	(<i>n</i> = 10) AD	<i>F</i>
<i>Agonum muelleri</i>	C	0	—	0	—	0.0 ± 0.02	0.3
<i>Amara crenata</i>	P	0	—	0	—	0.0 ± 0.01	0.1
<i>Amara montivaga</i>	P	0	—	0	—	0.0 ± 0.03	0.1
<i>Ancholeus nitidus</i>	C	0.0 ± 0.04	0.1	0	—	0	—
<i>Ancholeus puncticollis</i>	C	0	—	0.0 ± 0.01	0.1	0	—
<i>Anchomenus dorsalis</i>	C	0.6 ± 0.25	0.8	2.4 ± 1.06	1	0	—
<i>Badister unipustulatus</i>	C	0.0 ± 0.01	0.1	0	—	0	—
<i>Bembidion ambiguum</i>	C	0.1 ± 0.08	0.2	0	—	0	—
<i>Bembidion guttula</i>	C	0	—	0.0 ± 0.02	0.1	0	—
<i>Bembidion lampros</i>	C	0.5 ± 0.30	0.7	0.3 ± 0.20	0.3	0.7 ± 0.37	0.5
<i>Bembidion quadrimaculatum</i>	C	0.2 ± 0.10	0.4	0	—	0	—
<i>Brachinus crepitans</i>	C	0.0 ± 0.01	0.1	0.1 ± 0.04	0.4	0.0 ± 0.01	0.1
<i>Brachinus sclopetata</i>	C	0.1 ± 0.04	0.2	0.0 ± 0.02	0.2	0.2 ± 0.18	0.1
<i>Calathus ambiguus</i>	C	0	—	0.6 ± 0.43	0.5	0.0 ± 0.01	0.1
<i>Calathus fuscipes</i>	O	0	—	0	—	2.1 ± 0.89	0.9
<i>Calathus melanocephalus</i>	C	0	—	0	—	0.0 ± 0.01	0.1
<i>Calathus rotundicollis</i>	C	0	—	0	—	0.0 ± 0.01	0.2
<i>Calosoma maderae</i>	C	0.1 ± 0.05	0.3	0	—	0	—
<i>Carabus violaceus</i>	C	0	—	0	—	0.0 ± 0.02	0.1
<i>Clivina fossor</i>	C	0.0 ± 0.02	0.2	0.1 ± 0.03	0.3	0.1 ± 0.04	0.3
<i>Cylindera paludosa</i>	C	0	—	0.0 ± 0.01	0.1	0	—
<i>Harpalus atratus</i>	P	0.1 ± 0.06	0.1	0.0 ± 0.01	0.1	0	—
<i>Harpalus distinguendus</i>	O	0	—	0.3 ± 0.28	0.5	0.0 ± 0.03	0.1
<i>Harpalus serripes</i>	P	0	—	0	—	0.5 ± 0.48	0.1
<i>Harpalus sulphuripes</i>	P	0	—	0	—	0.0 ± 0.01	0.1
<i>Poecilus cupreus</i>	O	0.3 ± 0.32	0.3	3.0 ± 1.36	0.8	1.1 ± 0.50	0.9
<i>Poecilus purpurascens</i>	O	0	—	0	—	0.5 ± 0.45	0.1
<i>Pseudoophonus calceatus</i>	O	0	—	0.0 ± 0.01	0.1	0	—
<i>Pseudoophonus rufipes</i>	O	7.7 ± 2.80	1	4.4 ± 2.01	0.7	1.2 ± 0.36	1
<i>Pterostichus niger</i>	C	0.5 ± 0.44	0.4	0.0 ± 0.03	0.1	0.1 ± 0.04	0.4
<i>Pterostichus vernalis</i>	C	0	—	0	—	0.0 ± 0.01	0.1
<i>Syntomus obscuroguttatus</i>	C	0.0 ± 0.02	0.3	0	—	0	—
<i>Tachys bistratus</i>	C	0.0 ± 0.02	0.1	0.1 ± 0.05	0.2	0	—
<i>Trechus quadristriatus</i>	C	0.1 ± 0.04	0.3	0.0 ± 0.02	0.3	0	—

Mean seasonal captures (abundance, AD) (mean ± SE) and frequency (*F*) per trap. Species common to all regions are shown in bold. Trophic groups (TG) are carnivore (C), omnivore (O) or phytophage (P).

carabid community and their population fluctuations in a longer time-series. Sampling followed a similar methodology (two or three pitfall traps in each maize plot and three to eight sampling dates), although only the most abundant species were identified. Mean ± SD carabid abundance was calculated for each sampling date from May to October. The results were used to determine whether the composition and population peaks of dominant species could also be identified in a 2-year study such as this one.

Power analysis to determine required sample sizes

Prospective power analyses were carried out to determine sample sizes (number of paired sites of GM versus non-GM crop) needed to detect a change in carabid populations using a two tailed *t*-test (population decrease or increase). The probability of committing a type I error (α) was set at 0.05 and type II error (β) was set at 0.2 [statistical power = $(1 - \beta) = 0.8$]. The statistical power measures the chance of detecting an effect of a known magnitude

using a specified experimental design, and varies according to the magnitude of the effect, which was set at a 30% change regarding the comparator population, because this is considered adequate for studies on effects on nontarget organisms (Perry *et al.*, 2003; Lang & Bühler, 2012). Comparator populations were approximated by calculating average carabid abundance in each habitat type, within each region. Data were transformed by $\log_{10}(x + 1)$ when necessary for normalization. Power was calculated with JMP, version 11 (SAS Institute Inc., Cary, North Carolina).

Results

Carabids in maize fields

In the 30 maize fields sampled across the three study regions, 2368 individuals belonging to 34 species were collected. Table 1 shows mean abundance of each species per trap and week,

allowing comparison with captures in other studies. In general, the assemblages were species-poor and uneven, dominated (95% total catch) by a few very abundant species, characteristic of arable systems with a high level of disturbance. Species common to maize fields across the different study regions have the greatest potential for use as standardized indicators for monitoring impacts of GM maize. There were only seven species common to maize across the three regions; from most abundant to least, these were *Pseudoophonus rufipes* (DeGeer), *Poecilus cupreus* (L.), *Bembidion lampros* (Herbst), *Pterostichus niger* (Schaller), *Brachinus (Brachynidius) sclopeta* (Fabricius), *Clivina fossor* (L.) and *Brachinus crepitans* (L.). When aggregated into trophic groups, 70% of maize carabids were omnivores, 26% were obligate carnivores and only 4% were obligate phytophages.

Estimated species richness (Chao 1 mean \pm SD) was low, ranging from 18.5 ± 2.6 in Almacelles, 17.5 ± 1.3 in Bujaraloz and 25.0 ± 5.5 in La Seu. Shannon diversity index was 1.13 in Bujaraloz, 1.61 in Almacelles and 1.96 in La Seu. There were a few weak correlations between community measures and the abundance of single species or trophic groups, indicating that abundance of most species and trophic groups would be poor indicators of biodiversity. Only *B. lampros* and carnivores correlated with species richness (Pearson's correlation coefficient 0.43 and 0.5, respectively); Shannon index and *B. sclopeta* correlated positively and *P. rufipes* negatively (Pearson's correlation coefficient 0.4 and -0.42 , respectively).

Carabids in neighbouring habitats

Within each region, the similarity of carabid assemblages captured in maize compared with those captured in neighbouring habitats (field margins, alfalfa and semi-natural vegetation) was assessed to determine whether maize carabids were present in other habitats and where these could best be sampled. The highest number of shared species was observed between maize and field margins; 15, 12 and 14 species in Bujaraloz, Almacelles and La Seu, respectively. Nevertheless, similarity indices revealed that the most similar habitats were maize fields and their margins in Bujaraloz (0.94) and La Seu (0.96), whereas, in Almacelles (0.88), alfalfa was most similar to maize (0.90). The habitat least similar to maize was consistently the semi-natural vegetation, probably as a result of the lower perturbation of natural systems and the lower soil humidity compared with irrigated agricultural land; similarity indices were 0.7, 0.57 and 0.27, in Bujaraloz, Almacelles and La Seu, respectively. In maize field margins, 4938 individuals of 42 species were collected (see Supporting information, Appendix S1). In alfalfa, 1689 individuals of 30 species were collected (see Supporting information, Appendix S2). In semi-natural vegetation neighbouring maize plots, only 198 specimens were captured belonging to 30 species (see Supporting information, Appendix S3).

Contribution of habitat, region and year to overall variability

Habitat, region and year contributed to the baseline variability of the carabid data from maize agroecosystems in northeast Spain. Variance partitioning revealed that habitat identity accounted for the largest proportion of explained variability in the carabid

community, with distinct assemblages in each maize or alfalfa fields, in field margins and in semi-natural vegetation (Fig. 1). After habitat type, there were substantial regional (Bujaraloz versus Almacelles versus La Seu) and annual (2011 and 2012) differences, suggesting spatial and temporal turnover in the species pool. The main predictor (habitat, region or year) accounted for 100% of the variation in some cases indicating that the addition of further factors could not improve the explanatory capacity of the model. The estimated proportion of variability explained by the GLM model (D^2) was above 40% for all dependent variables but lower for the community measures (Fig. 1B,C).

When regional and year-to-year variation of the dependent variables was examined within each habitat, there were differences between regions (Fig. 2) and years in the abundance of most groups (overall carabids, omnivores and carnivores) and the seven ubiquitous maize species (*P. rufipes*, *P. cupreus*, *B. lampros*, *P. niger*, *B. sclopeta*, *C. fossor* and *B. crepitans*), although community measures were not sufficiently sensitive to reflect these differences. Differences between regions followed no general tendency in any of the three habitats considered, which is to be expected from three regions with differing agricultural contexts. In maize (Fig. 2A), overall carabid abundance was highest in Almacelles, possibly as a result of the higher weediness of maize. In field margins (Fig. 2B) and alfalfa (Fig. 2C), carabids were most abundant in Bujaraloz where landscape is more open than that of La Seu, and less altered than that of Almacelles. Omnivores were always the most abundant trophic group, followed by carnivores and phytophages.

Regarding year-to-year variation in maize, carabids were clearly more abundant in 2011 (100 ± 24.72) than in 2012 (70 ± 15.99) ($\chi^2 = 1863.3$, d.f. = 28, $P < 0.001$), a tendency followed by most groups and common species. Conversely, in field margins, carabids were more abundant in 2012 (233 ± 49.29) than in 2011 (164 ± 39.29) ($\chi^2 = 4144.7$, d.f. = 27, $P < 0.001$), and this was also true for omnivores and carnivores but not for phytophages. In alfalfa, there were no differences in carabid abundance between 2011 (78 ± 12.44) and 2012 (85 ± 20.09) ($\chi^2 = 1054.2$, d.f. = 22, $P = 0.058$) because omnivores were more abundant in 2012 and carnivores and phytophages in 2011.

Common carabids in maize fields

Considering historical data from 2005 to 2012, *P. rufipes* clearly dominated the assemblage in maize fields in Lleida; proportions were: 48% *P. rufipes*, 24% *P. cupreus*, 7% *Anchomenus dorsalis* (Pontoppidan), 4% *Bembidion* spp., 1% *Brachinus* spp. and 1% *Harpalus distinguendus* (Duftschmid). Mean abundance was very variable across fields and years (Fig. 3); however, the overall findings are similar to the results from Almacelles in 2011 and 2012, indicating that the identification of dominant species may not need prolonged sampling. The most abundant species, *P. rufipes*, *P. cupreus* and *A. dorsalis*, peaked around pollen-shed, indicating that this could be a good time for sampling.

Power: sample sizes required to detect GM effects

The sample sizes needed to detect a 30% carabid population change (pairs of maize fields or margins) are shown in

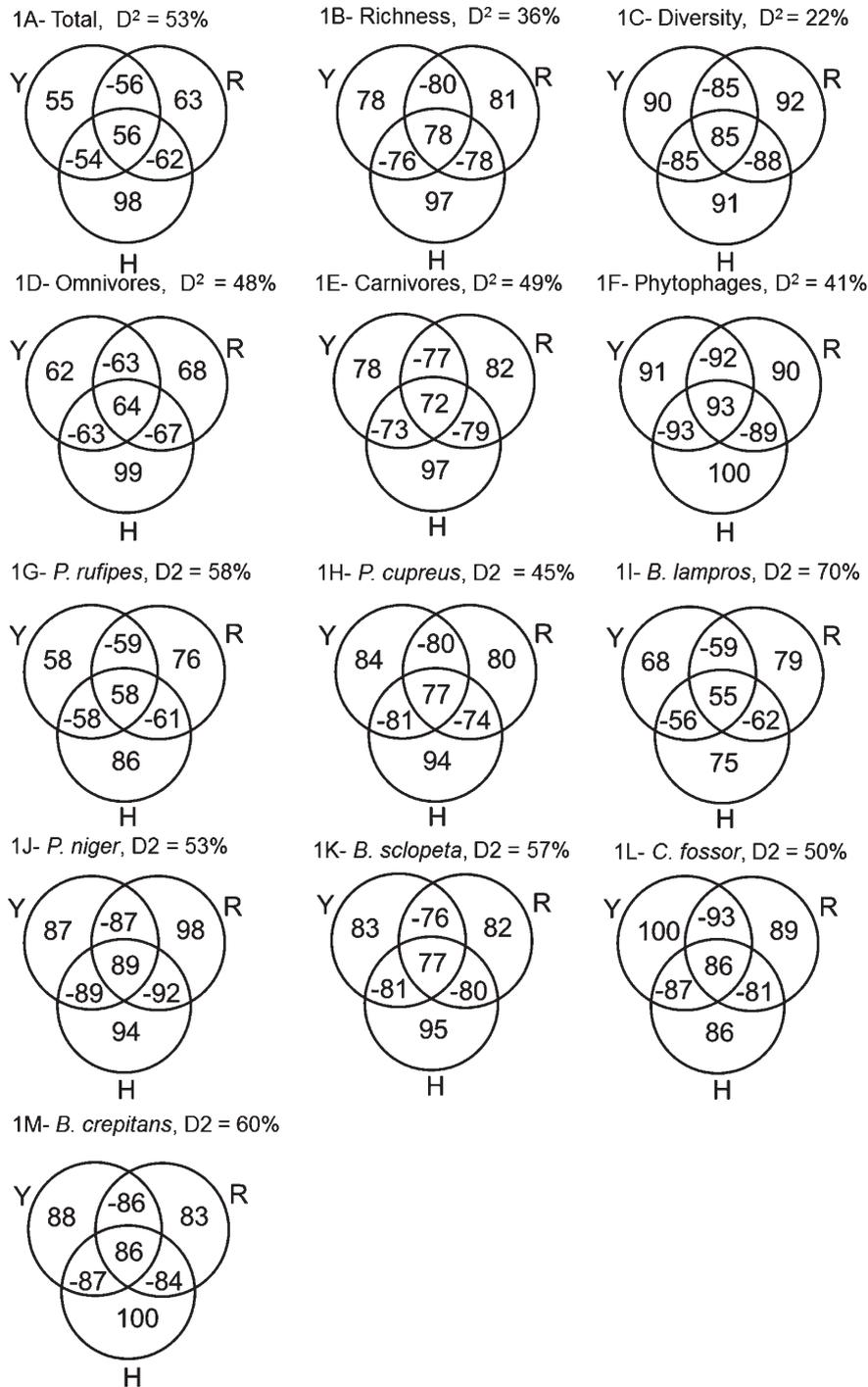


Figure 1 Variation partitioning of carabids among explanatory variables: region (R), habitat (H) and year (Y). The variability accounted for by the model is approximated by D^2 , and the proportion of variability explained by each factor and interaction between factors is shown for overall carabid number (A), Chao 1 estimated species richness (B), Shannon diversity index (C), trophic groups (D–F) and the seven ubiquitous species (G–M).

Table 2. Lowest sample sizes were obtained using overall carabid abundance (2–10 site pairs), closely followed by the observed (3–6 site pairs) or estimated (2–6 site pairs) species richness. When aggregated into trophic groups, sample sizes required were also low for omnivores (3–16 site pairs) and carnivores (9–46 site pairs) but very high for phytophages (77–1571 site

pairs), as a result of their relative rarity. Regarding single species, *P. rufipes* would require least site pairs (3–46 site pairs), followed by *P. cupreus* (26–394 site pairs) and *B. sclopeta* (33–884 site pairs). Other species required fewer samples but were not present in maize across regions. Sampling sizes to detect population change were generally lower in field margins than in maize

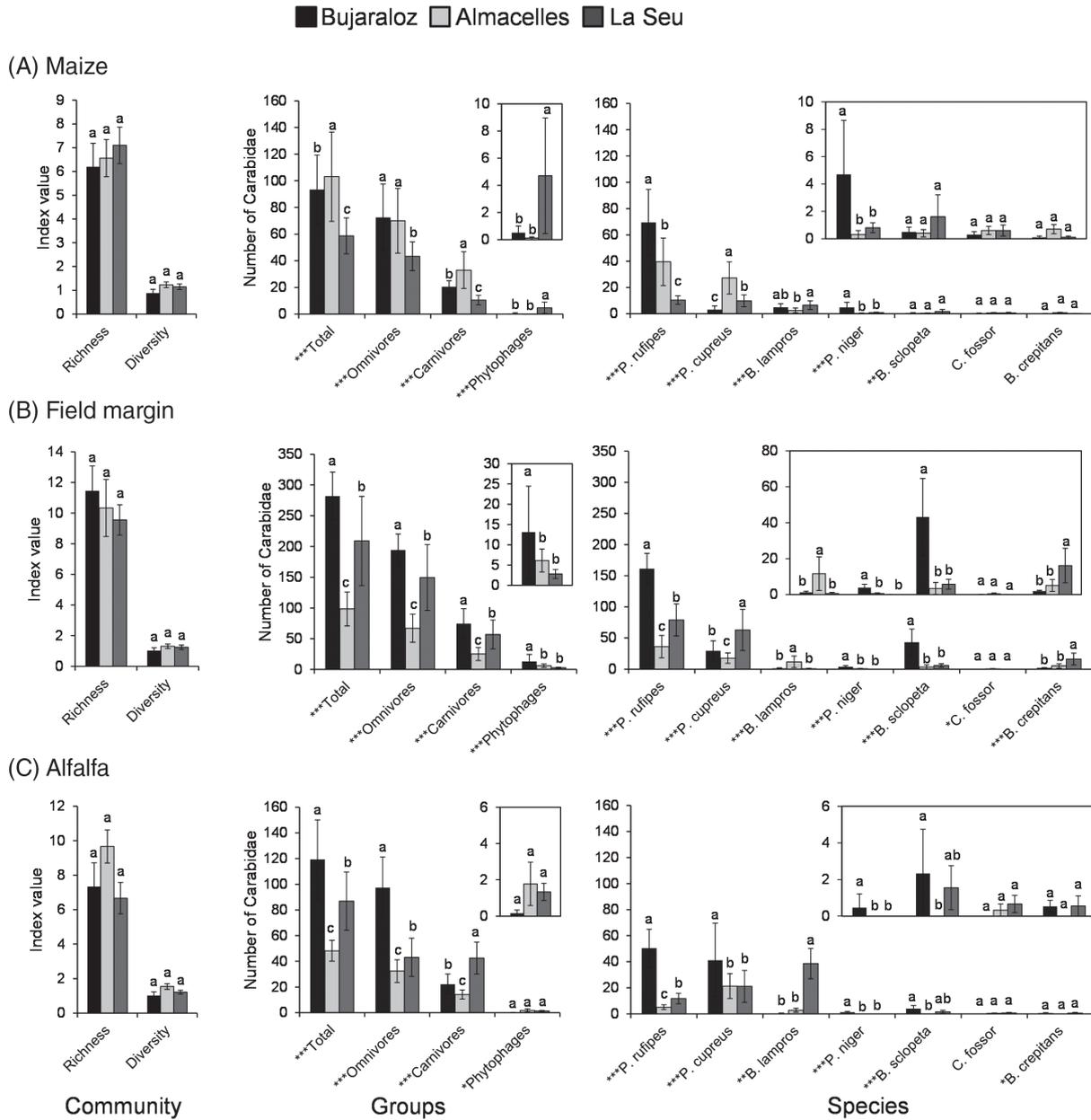


Figure 2 Regional (explanatory variable) differences between carabid community measures, abundance of groups and ubiquitous maize species (response variables on the x-axis) in three different habitats: maize (A), maize field margins (B) and alfalfa (C). Analysis was carried out using a generalized linear model, Poisson distribution and log-link function for count data, and Gaussian distribution and identity-link function for community measures. Differences were determined by a chi-squared test. Significant differences were explored by Tukey's honestly significant difference and are indicated by different lowercase letters; significance ($\alpha = 0.05$) is indicated as: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Degrees of freedom for community measures and abundance data, respectively, are: 23 and 24 for maize; 20 and 23 for margins; and 17 and 18 for alfalfa.

fields. In alfalfa (see Supporting information, Appendix S4), the lowest sample size to detect population change using *P. cupreus* was too high for a feasible monitoring plan.

Discussion

Unsurprisingly, species identity and abundance differed between regions, although they were located in the same geographical

area. This was an expected outcome because the study regions had been selected to represent agrienvironmental differences. For example, differences in the surrounding landscape in the three regions could have affected the composition of carabid populations (Vanbergen *et al.*, 2010). Similarly, differences in agricultural management in the three areas could have also influenced the composition and abundance of carabid populations (Holland & Luff, 2000). This highlights the need to test indicator species across a wide geographical area (Büchs, 2003) to ensure that

Table 2 Sample sizes (number of pairs of maize fields or margins) needed to detect a 30% change in carabid populations

Assessment endpoint	Bujaraloz		Almacelles		La Seu	
	Maize	Margin	Maize	Margin	Maize	Margin
All carabids	6	2	10	10	8	8
Diversity indices						
Richness (Sobs)	4	5	6	3	3	4
Richness (Chao 1)	5	5	6	2	3	4
Diversity (Shannon)	7	30	18	8	7	7
Trophic groups						
Omnivores	11	3	16	15	11	10
Carnivores	9	13	22	21	46	37
Phytophages	567	77	1571	143	253	103
Common maize species						
<i>Pseudophonus rufipes</i>	12	3	28	21	46	24
<i>Poecilus cupreus</i>	394	42	32	26	81	52
<i>Anchomenus dorsalis</i>	105	49	38	30	—	631
<i>Calathus fuscipes</i>	—	—	—	—	48	48
<i>Bembidion lampros</i>	117	267	394	951	120	84
<i>Calathus ambiguus</i>	—	1732	131	3078	1571	924
<i>Pterostichus niger</i>	—	164	1571	—	394	717
<i>Harpalus serripes</i>	—	—	—	—	253	271
<i>Poecilus purpurascens</i>	—	—	—	—	334	—
<i>Harpalus distinguendus</i>	—	269	319	107	1571	309
<i>Brachinus sclopeta</i>	394	33	884	121	771	318
<i>Bembidion quadrimaculatum</i>	394	1194	—	—	—	—
<i>Clivina fossor</i>	567	3078	394	—	615	1006

Calculations are based on the mean \pm SD for both 2011 and 2012 combined. Data were transformed by $\log_{10}(x + 1)$ where necessary for normalization. A pairwise *t*-test for independent data was used, assuming an equal number of units in each group and a two-tailed test, with statistical power set at 80% ($1 - \beta = 0.8$) and a significance level of $\alpha = 0.05$. Common maize species are ordered according to mean abundance. In bold, species from the maize field margins.

small differences in landscape, land management or climatic context do not reduce the efficacy of the indicator with respect to monitoring potential GM maize impacts. Conversely, despite differences in carabid abundance between years, there were few changes in the composition of the most abundant species (Ortego *et al.*, 2009; present study). In view of this, the most abundant or frequent species in a region may be identified in a few seasons if sampling a large number of sites.

The most cost-effective option is the monitoring of a few, easily identified taxa. The only species common to all regions, also frequent and abundant across sites, was *P. rufipes*. This species attains sufficient abundance that it is suited to be a focus of future monitoring because the number of sites required is realistic. Because it is also abundant in maize elsewhere in Europe (Smith *et al.*, 2008; Kocourek *et al.*, 2013), it could prove useful as an indicator across the EU (Büchs, 2003). Although *P. rufipes* is omnivorous, it is a good indicator of carabid biodiversity (Döring & Kromp, 2003) and is economically important, preying on invertebrates (Jørgensen & Toft, 1997) and weed seeds (Shearin *et al.*, 2008; Harrison & Gallandt, 2012). Populations of *P. rufipes* are correlated to larger spring-germinating weed seeds (Brooks *et al.*, 2012), the main weeds in maize. Reduced weed abundance as a long-term result of GMHT cropping could result in a drop in *P. rufipes* populations (Döring & Kromp, 2003; Eyre *et al.*, 2013). Because it reproduces and hibernates in the crop field (Luff, 1980), both adults and larvae are exposed to *Bt* toxins in soil. Finally, identification does not require great expertise because

it is a large species and there were no congeners leading to misidentification.

Using trophic group indicators allows quantification of the impact of changes in landscape or agricultural practices on ecological function (Purtauf *et al.*, 2005; Vanbergen *et al.*, 2010) despite differences in the identity of species. Phytophages may ingest toxins by feeding on *Bt* maize materials and they depend directly on resources provided by the weeds affected by cultivation of GMHT varieties. Moreover, they are very sensitive to environmental change (Purtauf *et al.*, 2005; Vanbergen *et al.*, 2010; Woodcock *et al.*, 2010). Nevertheless, the abundance of phytophage carabids was low, requiring sample sizes that were too high to be practicable for monitoring.

The main exposure pathway of carnivores to GM maize would be through prey (Meissle *et al.*, 2005). Because they are at the top of the trophic web, they integrate a substantial amount of ecological information from the maize community. They would be the best indicator of biodiversity and of invertebrate biological control function, and the sample sizes to detect differences would be sufficiently low for practical monitoring.

Omnivores are exposed through the mechanisms described for both phytophages and carnivores. Although they are considered to be less sensitive to environmental change than carnivores or phytophages (Purtauf *et al.*, 2005), they also respond to habitat alteration (Eyre *et al.*, 2013) and contribute to biological control. They were the most abundant trophic group and therefore sample sizes for detecting changes would be relatively smaller.

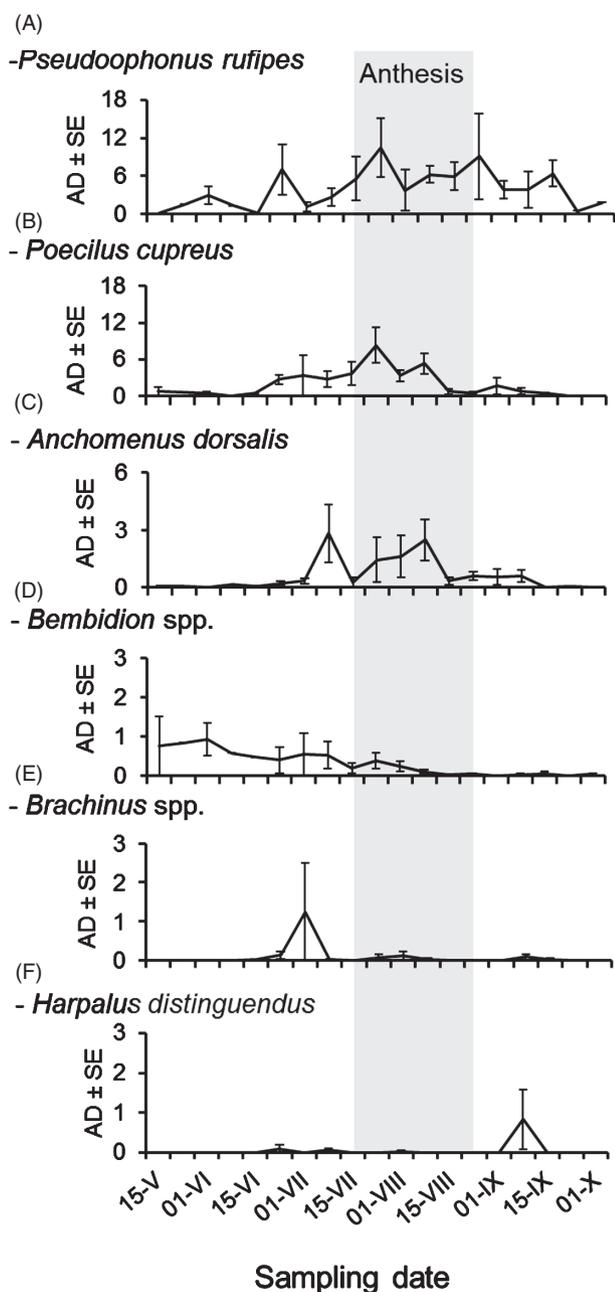


Figure 3 Population fluctuation of the most common carabid taxa (A–F) in Lleida plains during the maize growth cycle; the maize pollen-shed period is highlighted in grey. The graphs show weekly mean \pm SE captures per trap (abundance), calculated by pooling historical data from 2005 to 2012.

Carabid diversity measures are widely used as environmental indicators (Duelli & Obrist, 2003; Heink & Kowarik, 2010), as well as for detecting differences between farming systems (Holland & Luff, 2000). Indeed, in the present study, the lowest sample sizes were found using diversity measures, although these were unable to reflect differences in the identity and abundance of species existing between regions and years, possibly as a result of the low trap number and sampling window used per site. In these conditions, diversity measures are not sufficiently

sensitive for monitoring and they are not recommendable in PMEM.

Carabid communities are characteristic of each habitat (Smith *et al.*, 2008). Nevertheless, because maize shared many species with other habitats as recorded in the present study, as well as some others conducted in the area (Núñez, 1999; Madeira & Pons, 2015), impacts in GM maize fields would influence other communities. Indeed, species ubiquitous to maize were also present in field margins where sample sizes needed to detect changes were generally lower. Margins play an important role for carabid conservation in agricultural systems (Holland & Luff, 2000), as well as being closely exposed to GM cropping (Roy *et al.*, 2003; Bethwell *et al.*, 2012). Thus, exposure to *Bt* maize pollen deposition or increased herbicide drift as a result of GMHT maize cultivation could reduce carabid diversity or biological control functions in surrounding habitats. Finally, placement and access to traps is considerably easier than in maize fields.

Environmental monitoring is time consuming and costly and so the number of traps and sampling dates should be reduced when possible. The present study shows that using only three pitfall traps per sampling location could reveal population changes with low sample sizes for some species. The sampling date was not addressed specifically in the present study because it has recently been discussed in depth (Comas *et al.*, 2015). As in the present study, Comas *et al.* (2015) found that carabid catches were most abundant around maize pollen-shed. The relative variability decreased as the abundance of taxa increased, and so they recommended using sampling dates with the greatest abundance as long as the number of individual samples was sufficient to warrant a low sample size to detect changes in population numbers.

Conclusions and recommendations for monitoring

The present study contributes toward the design of a PMEM plan for detecting impacts of GMHT or *Bt* maize and, additionally, it provides baseline data on carabids that are valuable for monitoring the effects of natural or anthropogenic changes on maize agroecosystems. Overall, *P. rufipes* is the best indicator species, satisfying the criteria of abundance, relevance, sensitivity and ease of sampling. However, the carnivore group should also be included as indicator of invertebrate biological control and of biodiversity. The field margin is the best sampling location because this habitat is in close contact with the GM maize crops, it is exposed to GMHT and *GMBt* effects, and it shares many species with maize that are sufficiently abundant to require lower sample sizes to detect population changes. Finally, sampling should concentrate around pollen-shed because this is when carabid abundance is highest. The results obtained in the present study show that carabids may be useful for post-market environmental monitoring purposes and provide some recommendations for improving the practicability of PMEM without losing detection capacity.

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Supporting information

Additional Supporting information may be found in the online version of this article under the DOI reference: 10.1111/afe.12156

Appendix S1. Carabid species collected by pitfall trapping in margins of maize fields (n) in three regions of northeast Spain. Mean seasonal captures (abundance; AD) \pm SE and frequency (F) per trap are shown. Species common to all regions are shown in bold.

Appendix S2. Carabid species collected by pitfall trapping in alfalfa fields (n) in three regions of northeast Spain. Mean seasonal captures (abundance; AD) \pm SE and frequency (F) per trap are shown. Species common to all regions are shown in bold.

Appendix S3. Carabid species collected by pitfall trapping in semi-natural vegetation (n) in three regions of northeast Spain. Mean seasonal captures (abundance; AD) \pm SE and frequency (F) per trap are shown. Species common to all regions are shown in bold.

Appendix S4. Sample sizes (number of pairs of alfalfa fields) needed to detect a 30% change in carabid populations. Calculations are based on means and standard deviation for both 2011 and 2012 combined. Data were transformed by $\log(x + 1)$ where necessary for normalization. A pairwise t -test for independent data was used, assuming equal number of units in each group and two-tailed test, statistical power was set at 80% ($1 - \beta = 0.8$) and the significance level α was 0.05.

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