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SPECIAL ISSUE: MOLECULAR DETECTION OF TROPHIC INTERACTIONS

Predator community structure and trophic linkage strength to a focal prey

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Abstract

Predator abundance and community structure can affect the suppression of lower trophic levels, although studies of these interactions under field conditions are relatively few. We investigated how the frequency of consumption (measured using PCR-based gut content analysis) is affected by predator abundance, community diversity and evenness under realistic conditions. Soil arthropod communities in sixteen maize fields were measured (number of predators, diversity [Shannon H] and evenness [J]), and predator guts were searched for DNA of the focal subterranean herbivore, the corn rootworm (*Diabrotica virgifera*). Predator abundance and diversity were positively correlated with trophic linkage strength (the proportion positive for rootworm DNA), although the latter characteristic was not significantly so. The diversity and evenness of the predator community with chewing mouthparts were strongly correlated with their linkage strength to rootworms, whereas the linkage strength of fluid-feeding predators was unaffected by their community characteristics. Within this community, chewing predators are more affected by the rootworm's hemolymph defence. This research clearly shows that predator abundance and diversity influence the strength of a community's trophic linkage to a focal pest and that these community characteristics may be particularly important for less palatable or protected prey species. We also make the case for conserving diverse and abundant predator communities within agroecosystems as a form of pest management.

Keywords: biological control, *Diabrotica virgifera*, predator abundance, predator diversity, prey defence, soil food web

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Introduction

Biodiversity provides numerous services and is thus of both economic and societal value (Myers 1996; Hooper *et al.* 2005; Kumar *et al.* 2013). One service that may be contributed by healthy and diverse communities is pest management, specifically biological control of insect and weed pests by their natural enemies (Losey & Vaughan 2006; Finke & Snyder 2010). Predators, parasitoids and pathogens contribute to the natural suppression of pests in cropland and natural areas throughout the world, and biological control is conservatively estimated to save land managers billions of dollars annually by directly reducing pest populations and the input costs for managing these pests (Losey & Vaughan 2006; Landis *et al.* 2008; De Clercq *et al.* 2011). Pest populations and pest complexes represent a dynamic resource for predator communities (spatiotemporally and in quality), and it has been argued that conserving a diverse natural enemy community is important to the consistent suppression of changing prey populations (Cardinale *et al.* 2006; Douglass *et al.* 2008; Griffin & Silliman 2011). Yet as predator communities increase in abundance and diversity, food web theory (and experimental evidence) would predict the number and types of intraguild interactions (or other processes that disrupt predation) to increase, which could feasibly weaken specific trophic connections to a focal pest in need of management (Polis & Holt 1992; Ferguson & Stiling 1996; Finke & Denno 2004; Jonsson *et al.* 2007). However, most of the research designed to investigate

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these interactions have been in easily manipulated 'mesocosms', the results of which may or may not translate well to actual field conditions (O'Connor & Bruno 2009).

Molecular gut content analysis represents an important way to study the strength of trophic interactions with minimal disruption of an animal's natural feeding behaviour. Techniques that use a food's DNA as a food-specific marker can provide a clear picture of the diversity of organisms that consume a focal food item (Chen *et al.* 2000; Fournier *et al.* 2008; King *et al.* 2010; Eitzinger & Traugott 2011; Lundgren & Fergen 2011; Opatovsky *et al.* 2012). This is particularly useful in soil systems, where direct observation of predation events and use of sentinel, subterranean prey are challenging. Using gut content analysis and food-associated markers, a diversity of relative specific predator–prey linkages have been established to key agricultural pests in the soil (Chen *et al.* 2000; Symondson *et al.* 2000; Juen & Traugott 2007; Lundgren *et al.* 2009c, 2013; King *et al.* 2010), which has helped to support conservation biological control efforts. The use of gut content analysis to establish the relative trophic linkages of several arthropod *communities* to a focal pest has received much less attention than examining the relative strengths of *particular species* within a community to a focal food item (essentially identifying which members of the community are most important biological control agents). One motivation for focusing on the relative trophic linkages of different and entire predator communities is that this approach would allow measuring how communities representing a continuum in structure (abundance, diversity, evenness, etc.) respond trophically to a target pest.

The predator community of corn rootworm represents a good study system for exploring how a predator community's structure influences its relative trophic reliance on a focal prey species. Corn rootworms (specifically the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, Coleoptera: Chrysomelidae) costs farmers billions of dollars to control (Gray *et al.* 2009). Recent interest in conservation biological control of this pest has identified dozens of predators that consume the subterranean eggs and larvae of rootworms within North American and European maize fields (Lundgren *et al.* 2009b,c; Toepfer *et al.* 2009). Rootworm phenology, feeding behaviour within the root and defences have all been implicated in determining which of the predator complex are most influential in managing rootworms in the field, although clear keystone predators of this pest have not been identified. Perhaps more importantly, efforts have shown that conserving entire predator communities can reduce rootworm abundance and damage to maize roots (Lundgren & Fergen 2010, 2011), although the aspects of these predator communities that are contributing to rootworm management remain unexplored. The aim of our study was to test on a field-scale whether increasing predator community abundance, diversity and evenness results in stronger or weaker trophic linkages to a focal food, thereby helping to resolve whether predator diversity is a source or sink for biological control programmes under realistic conditions.

Materials and methods

Field sites

Research was conducted at the Eastern South Dakota Soil and Water Research Farm in 2009 and 2010 (44.3491, −96.8121; latitude, longitude). Eight no-till cornfields (18 × 24 m) were established in both study years in locations. Maize (Dekalb® hybrid DKC 44-92, Monsanto Company, St Louis Missouri, USA) was planted on 22 May 2009 and 21 May 2010. Seed was planted to a depth of 5 cm with 80 000 seeds ha^{-1} and 75 cm row spacing. Maize was preceded by soybeans, ensuring that rootworm populations were absent from the fields. At least 12 m of grassy margins separated the experimental fields. Herbicide was sprayed on each plot directly before planting to remove all vegetation. In 2009, 1.6 L ha^{-1} glyphosate (Roundup Weathermax®, Monsanto Company) and 2.3 L ha^{-1} S-metolachlor (Dual Magnum II®, Syngenta, Greensboro, North Carolina, USA) were sprayed on fields to control weeds; in 2010, 3.2 L ha^{-1} glyphosate (Roundup Weathermax) was used. Maize was fertilized at a rate of 84 kg ammonium nitrate ha^{-1} using a drop spreader on 9 July 2010.

Three patches in each field were infested with eggs of western corn rootworm on 23 April 2009 and 15 April 2010 (Sutter & Branson 1986). The three infestation levels were 3300, 1300 and 164 eggs row-m^{-1}. Each infestation level was randomly assigned to one quadrant of each field (the fourth quadrant was uninfested) and was situated in a central 6 × 3 m patch within that quadrat (thus, infested areas were 6 m from any other infested area of the plot). Infestations were conducted using a tractor-mounted rootworm egg infester (Sutter & Branson 1986). This approach ensured that a standardized series of rootworm infestations were present in every field.

Arthropod collections

Soil arthropod communities were collected from the soil column using soil cores. Arthropods within these cores move slowly among habitats relative to surface-dwelling arthropods (J. G. L., personal communication), reducing

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interfield movement of the sampled communities. Soil cores were collected on nine dates in each of 2009 (28 April, 11 May, 28 May, 11 June, 25 June, 1 July, 7 July, 14 July and 21 July) and 2010 (28 April, 10 May, 24 May, 7 June, 16 June, 24 June, 1 July, 8 July and 15 July) that covered the time period when rootworm immatures were present in the field. On each sample date, 12 cores (10 cm diameter and 10 cm deep) were collected from the base of randomly selected maize plants each field (three cores from each quadrant of the field). Soil from the cores in a field was pooled, and arthropods were extracted from the soil cores into 75% ethanol using Berlese funnels. The arthropod community was separated into two groups; those specimens (excluding corn rootworms) that escaped the soil in the first 24 h following collection were frozen at −20 °C until they could be processed for gut content analysis. The remaining specimens (extracted 2–7 d postcollection) were stored at room temperature for community analysis. Rootworm larvae were enumerated, and their head capsule widths and body lengths were recorded microscopically, thereby pinpointing their developmental stages (Hammack *et al.* 2003).

Predators were identified to the lowest taxonomic position possible for the samples whose guts were analysed. Exceptions were rove beetles (Coleoptera: Staphylinidae) that were grouped into three size categories, and spiders (Araneae), which were grouped at the ordinal level. Mites were excluded from the analysis. For the diversity analyses on the full community, ants, rove beetles and spiders were pooled at the Family (or Order) level because of a lack of taxonomic expertise. It is important to note that the diversity within these broader Family-level OTUs could vary differently from that of the overall community. For example, Staphylinidae (Balog *et al.* 2010) and spider (Uetz *et al.* 1999) communities in maize could each contain dozens of species. Much of the diversity found in the resulting communities was represented by Carabidae (17 of the 31 OTUs were carabids), a family which is represented by a wide range of feeding modes and diets. Furthermore, predator taxa were classified into chewing and fluid-feeding guilds based on author experience, relevant literature and discussions with experts in these groups.

Gut content analysis

The guts of each predator collected in the soil cores during the first 24 h postcollection were analysed using qPCR and rootworm-specific primer sets. Each specimen was dipped in 10% sodium hypochlorite solution for 10 s and then water prior to DNA extraction. The guts of taxa longer than 1 cm were dissected microscopically with sterilized instruments to reduce clogging of the extraction filters. It should be noted that preliminary trials showed that predators from collection vials with rootworm larvae did not have a significantly different detection level versus those predators from vials without rootworm larvae (J. G. L., unpublished data). DNA from individual specimens was extracted using DNEasy extraction kits for animal tissues (Qiagen, Valencia, CA) according to the kit instructions. Tissues were macerated in ATL buffer and incubated with proteinase K for 3 h. DNA extractions were then frozen at −20 °C until they could be analysed. Primer sets (fwd: 5-TAGTT CCCTTAATAATTGGTGCTC-3; rev: 5-CCCCCTTTCTA CTATCCTTCTTA-3) that specifically amplify a 119-bp fragment of the COI and partial tRNA-Leu genes of *Diabrotica virgifera virgifera* have been developed, screened for target specificity and were applied to the current experiment (Lundgren *et al.* 2009c; Lundgren & Fergen 2011). Each sample was amplified on a qPCR thermocycler (MX3000P, Stratagene, La Jolla, California) in 25-μL reaction mixtures containing 12.5 μL 2 × SYBR Green I Master Mix (Qiagen), 1 μL of template DNA, 1 μL each of forward and reverse primer sets (225 μM concentration) and 9.5 μL of PCR-grade water. The thermocycler programme used to amplify the rootworm DNA was 95 °C for 15 min, followed by 50 cycles of 94 °C for 15 s, 56 °C for 30 s and 72 °C for 30 s. Fluorescence was recorded at 492 nm (for SYBR Green) and 582 nm (for the ROX dye, used in verification of consistent reaction conditions) during the annealing step of each PCR cycle. Resulting fluorescence was adjusted manually to bring the baseline-corrected normalized fluorescence (dRn) just above background fluorescence for each plate. On each plate, five wells were devoted to positive controls (combined extractions from five third-instar rootworms), and three to no-template controls (i.e. PCR water). To verify amplicon identity, the melting temperature for each positive PCR product was determined by measuring the fluorescence continuously as the temperature was reduced from 95° to 55 °C at a rate of 0.2 °C s^{-1}. The proportion of specimens testing positive was determined for each field's community, as well as for each taxon in each field (pooled across the rootworm preimaginal development period).

Data analysis

The community metrics (e.g. the number of predators, diversity [H] and evenness [J]) were related to the proportion of predators positive for rootworm DNA for each field using linear regressions. The proportion of prey-positive chewing and sucking predators was correlated with their respective community abundance, diversities and evenness per field using linear regressions.

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Finally, relationships of community characteristics and trophic linkage strengths within specific predator taxa were compared with individual regressions. The effect of sample year on predator abundance, diversity and evenness per plot over the season was compared using independent ANOVAs. Within each year, the effect of sample dates on predator abundance, diversity and evenness per plot was compared using independent ANOVAs. All statistics were conducted with Systat 13 (Systat, Chicago, Illinois, USA).

Results

Arthropod community

Average ± SEM (range) seasonal total first, second and third instars collected per plot were 79.00 ± 33.28 (0–524), 6.5 ± 2.11 (0–27) and 9.63 ± 2.73 (0–34) larvae, respectively. Average total larvae per plot were 95.13 ± 35.77 (8–565). All larvae had pupated by 28 July in both study years. An average total of 20.13 ± 4.97 (1–69) adults were collected in emergence cages per plot.

For the diversity analysis, we grouped the predator community (N = 4352 specimens) into 31 operational taxonomic units (OTUs; Table 1), with averages (SEM) of 251.19 ± 24.66 specimens and 13.38 ± 0.60 OTUs collected per plot. Of these, rove beetles (N = 1154), ants (N = 875), predatory beetle larvae (mostly carabids; N = 647), japygids (N = 452) and *Polyderis* (N = 278) were the five most abundant taxa collected. Average diversity (Shannon H) and evenness (J) of these 16 communities were 1.91 ± 0.05 and 0.74 ± 0.01, respectively. There were significantly more predators collected in 2009 than in 2010, and predator diversity and evenness were similar between years (predator abundance: $F_{1,14}$ = 13.22, P = 0.003; Shannon index: $F_{1,14}$ = 0.44, P = 0.52; evenness: $F_{1,14}$ = 4.23, P = 0.06), thus reassuring that these experimental fields were independent in both space and time. Sample date within 2009 did not have a significant effect on predator abundance, diversity or evenness (predator abundance: $F_{8,63}$ = 1.60, P = 0.14; Shannon index: $F_{8,63}$ = 2.01; P = 0.06; evenness: $F_{8,63}$ = 1.11, P = 0.37). In 2010, predator abundance and diversity varied significantly over the sample period (predator abundance: $F_{8,63}$ = 7.02, P < 0.001;

Table 1 Mean ± SEM predatory operational taxonomic units (OTUs) discovered in 16 no-till maize fields infested with eggs of corn rootworms, and used in community diversity analyses

Order: Family	Operational taxonomic unit	Feeding style (C = chewing, F = fluid-feeding	Number of specimens per plot
Chilopoda	Centipede	F	11.69 ± 1.67 (16)
Myriapoda	Millipede	C	7.85 ± 2.05 (7)
Diplura: Japygidae	Japygid	F	28.25 ± 4.20 (16)
Pseudoscorpionida	Pseudoscorpion	F	8 ± 0 (1)
Opiliones: Phalangiidae	*Phalangium opilio* L.	C	1.33 ± 0.14 (3)
Araneae	Spider	F	9.19 ± 1.52 (16)
Coleoptera	Predatory beetle larvae	F	19.63 ± 1.42 (16)
Coleoptera: Anthicidae	Antlike flower beetle	C	1.75 ± 0.24 (4)
Coleoptera: Carabidae	*Agonum placidum* (Say)	C	1.6 ± 0.22 (5)
Coleoptera: Carabidae	*Amara apricaria* (Paykull)	C	1.5 ± 0.25 (4)
Coleoptera: Carabidae	*Anisodactylus discoideus* Dejean	C	3.2 ± 0.62 (5)
Coleoptera: Carabidae	*Bembidion rapidum* (LeConte)	C	1.50 ± 0.14 (4)
Coleoptera: Carabidae	*Clivina impressefrons* LeConte	C	3.42 ± 0.43 (12)
Coleoptera: Carabidae	*Dischirius globulosus* (Say)	C	1.67 ± 0.14 (3)
Coleoptera: Carabidae	*Discoderus parallelus*	C	1.33 ± 0.14 (3)
Coleoptera: Carabidae	*Elaphropus* sp.	C	11.07 ± 1.77 (15)
Coleoptera: Carabidae	*Polyderus* sp.	C	17.38 ± 3.30 (16)
Coleoptera: Carabidae	*Stenolophus comma* (Fabricius)	C	19.45 ± 5.78 (11)
Coleoptera: Coccinellidae	Lady beetle larva	F	3 ± 0.71 (2)
Coleoptera: Elateridae	Click beetle	C	1.25 ± 0.13 (4)
Coleoptera: Staphylinidae	Rove beetle	C	72.10 ± 6.34 (16)
Hymenoptera: Formicidae	Ant	F	54.69 ± 14.57 (16)
Orthoptera: Gryllidae	Cricket	C	5.67 ± 1.45 (9)

Additional taxa collected were represented by fewer than 3 specimens (<0.1% of the community). These taxa were an unidentified heteropteran and the carabids *Agonum lutulentum* (Leconte), *Bembidion quadrimaculatum* Say, *Discoderus parallelus* (Haldeman), *Harpalus herbivagus* Say, *Poecilus chalcites* (Say), *Stenolophus ochropezus* (Say) and an unidentified harpaline teneral (Coleoptera: Carabidae).

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Shannon index: $F_{8,63} = 8.12$; $P < 0.001$; Fig. S1, Supporting Information), but evenness was statistically consistent over the season ($F_{8,63} = 1.83$, $P = 0.09$).

Increasing plot-level prey abundance did not attract more diverse or more abundant predator communities. There was no relationship between pest larval abundance or third-instar abundance and predator abundance or diversity (Shannon H) in a plot (total larvae × predator abundance: $F_{1,14} = 1.06$, $P = 0.32$; total larvae × predator diversity: $F_{1,14} = 0.49$, $P = 0.50$; 3rd instar × abundance: $F_{1,14} = 2.56$, $P = 0.13$; 3rd instar × diversity: $F_{1,14} = 0.40$, $P = 0.54$).

Community-level predation metrics and diversity

A total of 1980 predator specimens were analysed for their consumption of rootworms, revealing an average frequency of consumption of 5.88 ± 0.69% per plot by the predator communities. Predator abundance was positively associated with frequency of consumption of rootworm immatures ($F_{1,14} = 6.17$, $P = 0.03$; Fig. 1a). Although there was a positive association between predator diversity and the frequency of consumption of rootworms, this relationship was not significant ($F_{1,14} = 2.74$, $P = 0.12$; Fig. 1b). There was no relationship of the community's evenness and linkage strength to rootworms ($F_{1,14} = 0.40$, $P = 0.89$).

Predator taxa within these communities responded differently towards rootworms as a result of increasing predator diversity (Table 2). Total predator abundance led to a significantly (or marginally significantly) higher frequency of rootworm consumption by *Solenopsis* subg. *Diplorhoptrum*, rove beetles >0.5 cm in length, *Polyderus* and *Stenolophus comma* (Table 2). Rootworm consumption by individual predator taxa was unaffected by community diversity and community evenness, although there was a marginally significant ($P < 0.1$) effect for japygids and predator diversity, and rove beetles (<0.5 cm in length) and species evenness.

Feeding guilds and trophic connections to rootworms

The two feeding guilds examined in this study responded very differently to the community structures in the experimental fields. Based on our categorical assignments of predators, the chewing predator community was 1.5-fold more species-rich than the fluid-feeding component of the communities (8.19 ± 0.62 and 5.19 ± 0.10 species per field), but fluid-feeding and chewing predators were similarly abundant (123.88 ± 17.83 versus 127.31 ± 12.04 specimens per field) and diverse (H-values of 1.33 ± 0.04 versus 1.29 ± 0.08) in the fields examined. The two feeding guilds consumed rootworms with equivalent overall

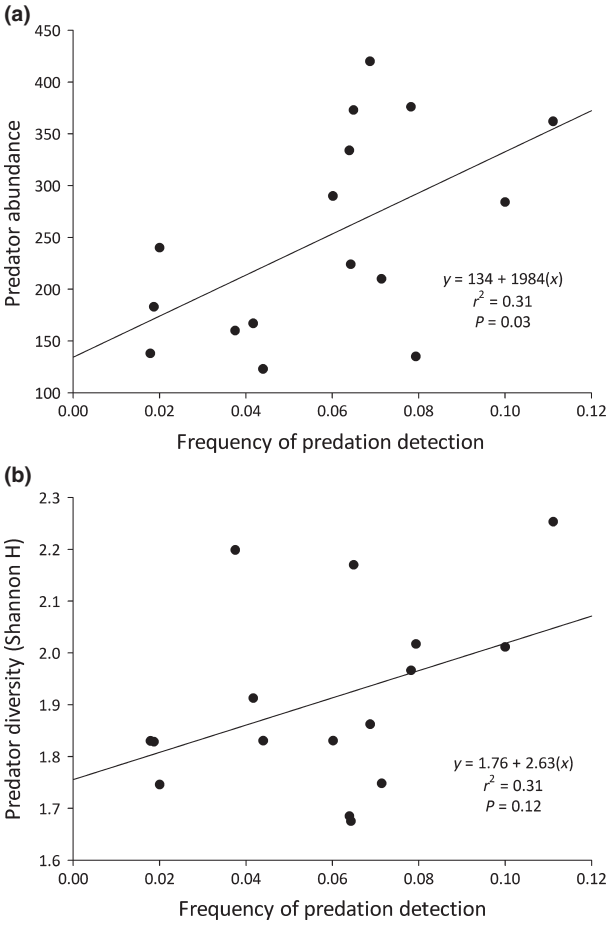

Fig. 1 Predator abundance (a) but not diversity (b) affects the proportion of predators testing positive for the consumption of western corn rootworm DNA. Each datapoint represents the entire predator community from a separate maize field, and the linear relationship was significant ($\alpha = 0.05$) for predator abundance, but not diversity. Additional statistics can be found in the text.

frequencies in the plots (6.32 ± 1.30 and 6.63 ± 1.02% of chewing and fluid-feeding predators tested positive for rootworm DNA). The trophic linkage of chewing predators to rootworms became stronger as the community became more diverse ($F_{1,14} = 8.80$, $P = 0.01$) and more even ($F_{1,14} = 7.01$, $P = 0.02$; Fig. 2), but not as the community became more abundant ($F_{1,14} = 0.87$, $P = 0.37$). In contrast, the trophic linkage of fluid-feeding predators was unaffected by the abundance, diversity or evenness of the fluid-feeding predator community (abundance: $F_{1,14} = 0.32$, $P = 0.58$; diversity: $F_{1,14} = 0.82$, $P = 0.38$; evenness: $F_{1,14} = 0.84$, $P = 0.37$).

Discussion

Abundance, diversity and evenness of a predator community often enhance its ability to respond to focal prey

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Table 2 The effects of predator abundance, diversity and evenness on the proportion of individual predator taxa testing positive for DNA of western corn rootworms in their stomachs. Significant ($\alpha = 0.05$) results are highlighted in grey, and marginally significant ($\alpha = 0.1$) interactions are emboldened and italicized

		Feeding style (C = chewing; F = fluid-feeding	Sample size (per n fields)	Abundance	Diversity	Evenness
Chilopoda	Centipede morpho 1	F	21 (10)	$F_{1,14} = 1.32$, $P = 0.27$	$F_{1,14} = 0.07$, $P = 0.79$	$F_{1,14} = 2.05$, $P = 0.17$
Diplura	Japygidae	C	200 (16)	$F_{1,14} = 0.05$, $P = 0.84$	$\boldsymbol{F_{1,14} = 3.76, P = 0.07}$	$F_{1,14} = 1.83$, $P = 0.20$
Coleoptera	Predatory beetle larvae	F	191 (16)	$F_{1,14} = 0.27$, $P = 0.61$	$F_{1,14} < 0.01$, $P = 0.96$	$F_{1,14} = 1.15$, $P = 0.30$
Col: Carabidae	*Clivina impressefrons*	C	19 (8)	$F_{1,14} = 0.24$, $P = 0.63$	$F_{1,14} = 0.01$, $P = 0.94$	$F_{1,14} = 0.44$, $P = 0.52$
Col: Carabidae	*Elaphropus* sp.	C	72 (14)	$F_{1,14} = 0.86$, $P = 0.37$	$F_{1,14} = 0.30$, $P = 0.60$	$F_{1,14} = 0.13$, $P = 0.73$
Col: Carabidae	*Polyderus* sp.	C	153 (15)	$\boldsymbol{F_{1,14} = 3.43, P = 0.09}$	$F_{1,14} = 0.02$, $P = 0.91$	$F_{1,14} = 2.77$, $P = 0.12$
Col: Carabidae	*Stenolophus comma*	C	50 (8)	$F_{1,14} = 6.62$, $P = 0.02$	$F_{1,14} = 2.78$, $P = 0.12$	$F_{1,14} = 0.23$, $P = 0.64$
Col.: Staphylinidae	Rove beetle (>1 cm)	C	13 (3)	$F_{1,14} = 5.36$, $P = 0.04$	$F_{1,14} = 0.04$, $P = 0.85$	$F_{1,14} = 1.74$, $P = 0.21$
Col.: Staphylinidae	Rove beetle (0.5–1 cm)	C	55 (14)	$\boldsymbol{F_{1,14} = 3.66, P = 0.08}$	$F_{1,14} = 0.08$, $P = 0.78$	$F_{1,14} = 1.34$, $P = 0.27$
Col.: Staphylinidae	Rove beetle (<0.5 cm)	C	716 (16)	$F_{1,14} = 0.99$, $P = 0.34$	$F_{1,14} = 0.97$, $P = 0.34$	$\boldsymbol{F_{1,14} = 3.72, P = 0.07}$
Hymenoptera: Formicidae	*Ponera pennsylvanicus*	F	194 (15)	$F_{1,14} = 0.91$, $P = 0.36$	$F_{1,14} = 1.87$, $P = 0.19$	$F_{1,14} < 0.01$, $P = 0.95$
Hym.: Formicidae	*Formica* sp.	F	177 (7)	$F_{1,14} = 0.64$, $P = 0.44$	$F_{1,14} = 0.14$, $P = 0.71$	$F_{1,14} = 0.60$, $P = 0.45$
Hym: Formicidae	*Solenopsis* subg. *Diplorhoptrum*	F	50 (6)	$F_{1,14} = 8.37$, $P = 0.01$	$F_{1,14} = 0.15$, $P = 0.71$	$F_{1,14} = 2.60$, $P = 0.13$

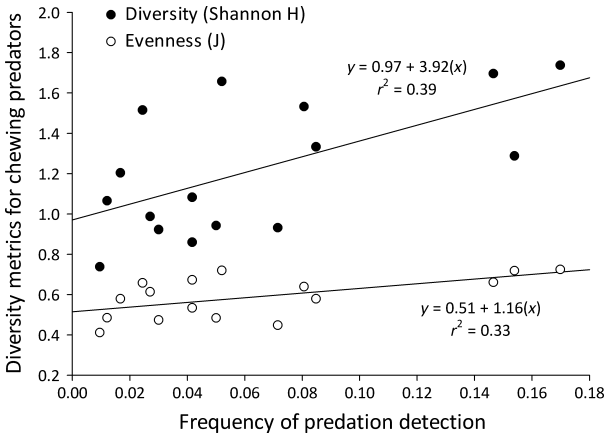

Fig. 2 Predator diversity (Shannon H) and evenness (J) affect the linkage strength of chewing predators and western corn rootworms. Linkage strength refers to the frequency of detection of rootworm DNA with predator guts. Each datapoint represents the component of the predator community with chewing mouthparts in a separate maize field. Both linear relationships are significant ($\alpha = 0.05$), and additional statistics can be found in the text.

(Douglass *et al*. 2008; Crowder *et al*. 2010; Lundgren & Fergen 2011). Our data support this notion by clearly showing that trophic linkages of a predator community to a target prey species are either unaffected or enhanced (but never negatively affected) as the diversity, evenness and abundance of the predator community increase. Within these overall predator communities, the relationships between the frequency with which individual taxa ate rootworms and the abundance, diversity and evenness were seldom strong (only 3 of 39 interactions were significant, and these significant taxa were captured in fewer than half of the fields; Table 2). In other words, the correlations between predator abundance, diversity and evenness and the trophic behaviour of the overall predator community was not driven by strong interactions in the feeding behaviour of individual predator species, but rather the predator species had an additive response in their consumption of rootworms that correlated with community patterns. Several aspects of the implementation of this experiment may have affected the observed outcomes. For example, although we focused on predators within the soil column (rather than those on the soil surface which arguably have higher mobility) and enclosed each field with a grass buffer strip, interfield movement of predators may have affected the relative abundance of specific taxa in our study. Also, different predator taxa, or even individuals within a taxon, digest food at different rates and could have affected the frequency of food DNA detection. By using a relatively large sample size (nearly 2000 predators were analysed) and examining

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entire predator communities that each represents a continuum of digestion efficiencies, we overcame spurious results driven by particularly strong or weak signatures of individual predators. Despite these potential shortcomings of the approaches used, our data support the idea that conservation programmes should focus on abundant and diverse predator communities that can dynamically respond to shifting conditions in target prey, rather than focusing on conserving specific predator species within a habitat.

It is important to note that this experiment did not measure a positive aggregation response of predators to increasing rootworm densities. Thus, the most abundant and diverse predator communities were not associated with the most abundant pest populations, which would render our conclusions misleading. Rather, we believe that the increasing trophic linkage (or functional response) to the pest by the endemic community of predators is the result of increasing food limitation and greater niche complementarity in the more abundant and diverse predator communities rather than a numerical response to prey density. Predators were free to immigrate and emigrate from the study systems as necessary to accommodate resource needs. This suggests that predators within the soil column may shift their diet to fully exploit local resources rather than move to an area with higher quality or more preferred prey (Lundgren & Harwood 2012).

Our observations on the interactions of predator feeding behaviour and community characteristics were particularly true when we considered the relative effectiveness of a prey's defence against different predator feeding guilds within the community. We hypothesize that rootworm prey are unpreferred by the predator community and that predator abundance and diversity help to force members of this predator community to include rootworms as part of their diet. As a predator community becomes more abundant, it is feasible that preferred prey will often become exploited, and they will rely more on less preferred food (Griffin *et al.* 2008; Griffiths *et al.* 2008). Furthermore, as a predator community diversifies, the number of dietary niches of this community might be predicted to expand to include prey species less preferred by a more taxonomically constricted predator community (Tilman *et al.* 1997; Loreau 1998; Wilby *et al.* 2005; Finke & Snyder 2010). This could also include intraguild prey, which is theorized to detract from predation on a target food. Rootworm larvae are hidden within maize roots during much of their lives (Moeser & Hibbard 2005) and have an antipredator hemolymph defence (Lundgren *et al.* 2009a, 2010); both traits restrict predator exposure to and preferences for rootworms. Specifically, fluid-feeding, or sucking, predators are less inhibited by the rootworm's defence than chewing predators are (Lundgren *et al.* 2009c, 2010). Gut content analysis of the predator community revealed that more abundant predator communities had a stronger trophic linkage to rootworms than less abundant communities (Fig. 1), which is in line with the hypothesis that predator abundance shifts predator reliance to this less preferred prey. When we examined these two feeding guilds separately, rootworm consumption by fluid-feeding predators was entirely unaffected by predator diversity or abundance, potentially because this prey is relatively preferred by sucking predators compared with chewing predators. In stark contrast, both diversity and evenness of the chewing predator community were strongly and positively correlated with the frequency at which the chewing predator community consumed rootworms (Fig. 2), suggesting that less diverse communities are preferentially eating foods other than rootworms. All of this is to say that predator community abundance and diversity may be particularly important to consider when implementing conservation biological control programmes of cryptic or defended prey items, which include many pests (Finke & Snyder 2010).

All of this begs the question of whether the increases in consumption frequency by more abundant and diverse communities observed in this study are meaningful for biological control of this important pest. While we cannot draw conclusions on this question from the given data set, some insight can be gained from other recent work where gut content analysis was used to study predator communities of the rootworm. Here, frequency of rootworm consumption based on gut content analysis effectively measured the diversity of predators relying on a target food item (Lundgren *et al.* 2009b,c; Lundgren & Fergen 2011). However, frequency of detection on its own did not scale well with predation rates on sentinel rootworm larvae, or reductions in rootworm populations or damage to maize roots. Combining frequency of detection with the quantity of rootworm DNA found in the predator stomach to generate a predation index was significantly associated with third-instar rootworm populations and root damage (Lundgren & Fergen 2011). Nevertheless, traditional PCR, not qPCR, remains the tool used by nearly all published literature to establish trophic interactions to a focal pest. To conclude, although trophic interaction strength of the predator community increased with predator abundance and diversity, additional work on how predator diversity scales with other metrics of predator efficacy (e.g. prey removal rates, prey population levels, pest damage) would help to strengthen the case for increasing predator diversity in cropland.

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References

Balog A, Kiss J, Szekeres D, Szenasi A, Marko V (2010) Rove beetles (Coleoptera: Staphylinidae) communities in transgenic Bt (MON810) and near isogenic maize. *Crop Protection*, **29**, 567–571.

Cardinale BJ, Srivastava DS, Duffy JE *et al.* (2006) Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature*, **443**, 989–992.

Chen Y, Giles KL, Payton ME, Greenstone MH (2000) Identifying key cereal aphid predators by molecular gut analysis. *Molecular Ecology*, **9**, 1887–1898.

Crowder DW, Northfield TD, Strand MR, Snyder WE (2010) Organic agriculture promotes evenness and natural pest control. *Nature*, **466**, 109–112.

De Clercq P, Mason P, Babendreier D (2011) Benefits and risks of exotic biological control agents. *BioControl*, **56**, 681–698.

Douglass JG, Duffy JE, Bruno JF (2008) Herbivore and predator diversity interactively affect ecosystem properties in an experimental marine community. *Ecology Letters*, **11**, 598–608.

Eitzinger B, Traugott M (2011) Which prey sustains cold-adapted invertebrate generalist predators in arable land? Examining prey choices by molecular gut-content analysis. *Journal of Applied Ecology*, **48**, 591–599.

Ferguson KI, Stiling P (1996) Non-additive effects of multiple natural enemies on aphid populations. *Oecologia*, **108**, 375–379.

Finke DL, Denno RF (2004) Predator diversity dampens trophic cascades. *Nature*, **429**, 407–410.

Finke DL, Snyder WE (2010) Conserving the benefits of predator biodiversity. *Biological Conservation*, **143**, 2260–2269.

Fournier V, Hagler JR, Daane K, de Leon J, Groves R (2008) Identifying the predator complex of *Homalodisca vitripennis* (Hemiptera: Cicadellidae): a comparative study of the efficacy of an ELISA and PCR gut content assay. *Oecologia*, **157**, 629–640.

Gray ME, Sappington TW, Miller NJ, Moeser J, Bohn MO (2009) Adaptation and invasiveness of western corn rootworm: intensifying research on a worsening pest. *Annual Review of Entomology*, **54**, 303–321.

Griffin JN, Silliman BR (2011) Predator diversity stabilizes and strengthens trophic control of a keystone grazer. *Biology Letters*, **7**, 79–82.

Griffin JN, de la Haye KL, Hawkins SJ, Thompson RC, Jenkins SR (2008) Predator diversity and ecosystem functioning: density modifies the effect of resource partitioning. *Ecology*, **89**, 298–305.

Griffiths GJK, Wilby A, Crawley MJ, Thomas MB (2008) Density-dependent effects of predator species-richness in diversity-function studies. *Ecology*, **89**, 2986–2993.

Hammack L, Ellsbury MM, Roehrdanz RL, Pikul JL Jr (2003) Larval sampling and instar determination in field populations of northern and western corn rootworm (Coleoptera: Chrysomelidae). *Journal of Economic Entomology*, **96**, 1153–1159.

Hooper DU, Chapin FS III, Ewel JJ *et al.* (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, **75**, 3–35.

Jonsson M, Johansson F, Karlsson C, Brodin T (2007) Intermediate predator impact on consumers weakens with increasing predator diversity in the presence of a top-predator. *Acta Oecologica*, **31**, 79–85.

Juen A, Traugott M (2007) Revealing species-specific trophic links in soil food webs: molecular identification of scarab predators. *Molecular Ecology*, **16**, 1545–1557.

King RA, Vaughan IP, Bell JR, Bohan DA, Symondson WOC (2010) Prey choice by carabid beetles feeding on an earthworm community analysed using species- and lineage-specific PCR primers. *Molecular Ecology*, **19**, 1721–1732.

Kumar P, Brondizio E, Gatzweiler F *et al.* (2013) The economics of ecosystem services: from local analysis to national policies. *Current Opinion in Environmental Sustainability*, **5**, 78–86.

Landis DA, Gardiner MM, van der Werf W, Swinton SM (2008) Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes. *Proceedings of the National Academy of Sciences*, **105**, 20552–20557.

Loreau M (1998) Biodiversity and ecosystem functioning: a mechanistic model. *Proceedings of the National Academy of Sciences of the USA*, **95**, 5632–5636.

Losey JE, Vaughan M (2006) The economic value of ecological services provided by insects. *BioScience*, **56**, 311–323.

Lundgren JG, Fergen JK (2010) The effects of a winter cover crop on *Diabrotica virgifera* (Coleoptera: Chrysomelidae) populations and beneficial arthropod communities in no-till maize. *Environmental Entomology*, **39**, 1816–1828.

Lundgren JG, Fergen JK (2011) Enhancing predation of a subterranean insect pest: a conservation benefit of winter vegetation in agroecosystems. *Applied Soil Ecology*, **51**, 9–16.

Lundgren JG, Harwood JD (2012) Functional responses to food diversity: the effect of seed availability on the feeding behavior of facultative granivores. *Journal of Entomological Science*, **47**, 160–176.

Lundgren JG, Haye T, Toepfer S, Kuhlmann U (2009a) A multi-faceted hemolymph defense against predation in *Diabrotica virgifera virgifera* larvae. *Biocontrol Science and Technology*, **19**, 871–880.

Lundgren JG, Nichols S, Prischmann DA, Ellsbury MM (2009b) Seasonal and diel activity patterns of generalist predators associated with *Diabrotica virgifera* immatures (Coleoptera: Chrysomelidae). *Biocontrol Science and Technology*, **19**, 327–333.

Lundgren JG, Prischmann DA, Ellsbury MM (2009c) Analysis of the predator community of a subterranean herbivorous insect based on polymerase chain reaction. *Ecological Applications*, **19**, 2157–2166.

Lundgren JG, Toepfer S, Haye T, Kuhlmann U (2010) Hemolymph defence in an invasive herbivore: its breadth of effectiveness against predators. *Journal of Applied Entomology*, **134**, 439–448.

Lundgren JG, Saska P, Honěk A (2013) Molecular approach to describing a seed-based food web: the post-dispersal granivore community of an invasive plant. *Ecology and Evolution*, **3**, 1642–1652.

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Moeser J, Hibbard BE (2005) A synopsis of the nutritional ecology of larvae and adults of *Diabrotica virgifera virgifera* (LeConte) in the new and old world- nouvelle cuisine for the invasive maize pest *Diabrotica virgifera virgifera* in Europe? In: *Western Corn Rootworm: ecology and management* (eds Vidal S, Kuhlmann U. & Edwards C R), pp. 41–67. CABI Publishing, Wallingford, UK.

Myers N (1996) Environmental services of biodiversity. *Proceedings of the National Academy of Sciences of the USA*, **93**, 2764–2769.

O'Connor MI, Bruno JF (2009) Predator richness has no effect in a diverse marine food web. *Journal of Animal Ecology*, **78**, 732–740.

Opatovsky I, Chapman EG, Wientraub PG, Lubin Y, Harwood JD (2012) Molecular characterization of the differential role of immigrant and agrobiont generalist predators in pest suppression. *Biological Control*, **63**, 25–30.

Polis GA, Holt RD (1992) Intraguild predation: the dynamics of complex trophic interactions. *Tree*, **7**, 151–154.

Sutter GR, Branson TF (1986) Artificial infestation of field plots. In: *Methods for the Study of Pest Diabrotica* (eds Krysan J L & Miller T A), pp. 147–157. Springer-Verlag, New York, NY.

Symondson WOC, Glen DM, Erickson ML, Liddell JE, Langdon CJ (2000) Do earthworms help to sustain the slug predator *Pterostichus melanarius* (Coleoptera: Carabidae) within crops? Investigations using monoclonal antibodies. *Molecular Ecology*, **9**, 1279–1292.

Tilman D, Knops JMH, Wedin D *et al.* (1997) The influence of functional diversity and composition on ecosystem processes. *Science*, **277**, 1300–1302.

Toepfer S, Haye T, Erlandson M *et al.* (2009) A review of the natural enemies of beetles in the subtribe Diabroticina (Coleoptera: Chrysomelidae): implications for sustainable pest management. *Biocontrol Science and Technology*, **19**, 1–65.

Uetz GW, Halaj J, Cady AB (1999) Guild structure of spiders in major crops. *Journal of Arachnology*, **27**, 270–280.

Wilby A, Villareal SC, Lan LP, Heong KL, Thomas MB (2005) Functional benefits of predator species diversity depend on prey identity. *Ecological Entomology*, **30**, 497–501.

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Data accessibility

All rootworm and predator data generated in arthropod community analysis: Dryad doi:10.5061/dryad.c2n18.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Seasonal changes in predator abundance and Shannon index (H) in eight predator communities collected in 2010 maize fields.

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