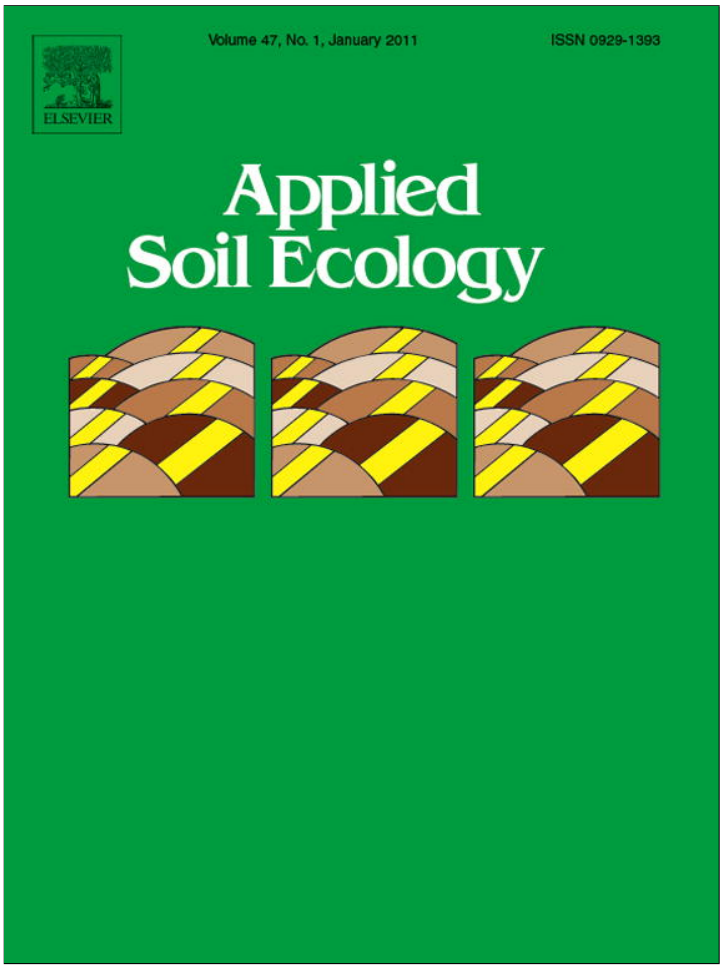

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Applied Soil Ecology 51 (2011) 9–16

Contents lists available at SciVerse ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Enhancing predation of a subterranean insect pest: A conservation benefit of winter vegetation in agroecosystems

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ARTICLE INFO

Article history:
Received 16 May 2011
Received in revised form 17 August 2011
Accepted 19 August 2011

Key words:
Biological control
Cover crops
Diabrotica virgifera
Gut content analysis
Soil food webs
Trophic interactions

ABSTRACT

Generalist predator communities are abundant and diverse in agroecosystems, but pests often persist nevertheless. Winter vegetation (e.g., cover crops) provides an agronomically sound opportunity to conserve predator communities and promote their impact on pests. We evaluate whether winter vegetation increases predation of *Diabrotica virgifera*, a key subterranean pest of maize. Fields of maize were preceded by a winter cover crop (slender wheatgrass) or a fallow period (bare soil) over two years. Pest populations and root damage were measured in each field, from which the gut contents of predators aspirated from the soil surface, or extracted from the soil column, were analyzed using qPCR and primer sets specific to *D. virgifera* COI gene sequences. Predation intensity on restrained *D. virgifera* larvae (sentinels) was observed during the three larval stadia of the pest ($n = 400$ 3rd instars per plot per stadium). A diverse predator community consumed *D. virgifera* in maize fields, and predation was significantly greater in maize following cover crops (as measured with sentinels, but not gut content analysis). Predation was particularly intense during the 3rd stadium of the pest, especially in the cover-cropped maize. qPCR-based gut content analysis of natural populations functioned well in determining which predators consumed *D. virgifera*, but was only correlated with their impact on the pest and its damage when the relative frequency of detection, quantity of DNA calculated, and predator abundance were combined into a predation index. In support of these observations, predation intensity on sentinels was negatively correlated with *D. virgifera* populations and plant damage, but did not provide an accurate picture of the community involved. Cover crops reduced *D. virgifera* populations by increasing predation levels on this pest, which indicates that conserving predation as an ecosystem service is a mechanism for how this form of habitat diversification functions. Also, we conclude that employing diverse methods provides the best insight into trophic relationships within subterranean systems. Finally, because of the dynamic and diverse interactions between pests and their natural enemy complexes, we advocate conserving diverse predator communities within agroecosystems, rather than targeting conservation efforts at specific key predator taxa.

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1. Introduction

Trophic relationships within soil food webs have important implications for both above and belowground terrestrial ecosystem processes (Wardle et al., 2005; Fountain et al., 2008; Eisenhauer et al., 2009), yet we know very little about the key interactions within subterranean food webs (Bardgett, 2002; Coleman, 2008; van der Putten et al., 2009; Nielsen et al., 2010). Top-down factors (i.e., predation, parasitism, and disease) influence biological communities, and these processes can be conserved within a habitat to reduce pestiferous species through biological control (Symondson et al., 2002; Snyder et al., 2006; Macfadyen et al., 2009). Although predator populations are diverse and abundant even in intensively managed agroecosystems, pests persist and the question remains as to how we can promote predator services without sacrificing farm productivity. Central to understanding this question is realizing that predators evolved within natural systems that are relatively undisturbed and biodiverse compared with ephemeral cropland (Tscharntke et al., 2007; Macfadyen and Bohan, 2010). Within these natural systems, predators rely on numerous resources (prey and non-prey foods, overwintering sites, favorable microclimates, preferred oviposition sites, etc.) that are often reduced or removed in annual cropping systems (Landis et al., 2000; Lundgren, 2009). Conserving ecosystem characteristics that support predator function to cropland while maintaining farm profitability is challenging. A practice currently advocated in sustainable agriculture that has repeatedly been shown to increase predator abundance is the deployment of winter (often non-crop) vegetation, or cover cropping. In addition to the numerous agronomic benefits of cover cropping to soil health and weed

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0929-1393/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.apsoil.2011.08.005

suppression (Clark, 1998), cover crops often reduce insect pest pressure in the subsequent crop (Brust and House, 1990; Bugg and Waddington, 1994; Tillman et al., 2004). The precise mechanisms for why these patterns occur remain largely unstudied, especially in soil food webs which are likely directly affected by the additional complexity that winter cover crops and their residue provide to this habitat. Development of new tools for unraveling subterranean trophic linkages between complex predator communities and agricultural pests, and for promoting the ecosystem services of predators in cropland, will make the application of biologically based pest management more realistic for land managers.

Studying soil food web interactions is difficult without disrupting normal community processes, and the best picture of subterranean trophic dynamics will likely come from simultaneously employing several methodological approaches (Luck et al., 1988; Harwood and Obrycki, 2005; Weber and Lundgren, 2009a). Quantifying predator communities that co-occur with a target pest is important in determining which species are putative natural enemies, but population monitoring provides little information on which predators are consuming the prey of interest. Predation intensity measured with sentinel prey items (i.e., known numbers of prey emplaced in a habitat and subsequently recollected) identifies which predators find the target prey acceptable in the field, but the precise natural predator–prey dynamics are difficult to recreate using this method (Muilenburg et al., 2008; Lundgren et al., 2010). Gut content analysis of predators is useful for identifying specific trophic linkages within a food web (i.e., knowing which species are eating a target species, less the species not focused on like birds or rodents) (Juen and Traugott, 2007; Fournier et al., 2008; Kuusk et al., 2008; Harwood et al., 2009; King et al., 2010), but there remain strong concerns regarding the correlation of predation intensity on a pest and the feeding indices provided by gut content analysis (Naranjo and Hagler, 2001; Harwood and Obrycki, 2005; Greenstone et al., 2007; Weber and Lundgren, 2009b). Given that all predation metrics have caveats to their interpretation, it is currently unknown for most systems which metrics are best correlated with predator function in the sense of biological control of a given prey type.

Diabrotica virgifera virgifera (Coleoptera: Chrysomelidae) is a subterranean pest of maize roots (Vidal et al., 2005; Gray et al., 2009) whose suppression may benefit from farm management efforts that conserve its natural enemy community. The fact that this insect incurs 95–99% mortality prior to eclosion (Onstad et al., 2006; Hibbard et al., 2010) suggests that predation by the abundant predator community of this insect is intense (Lundgren et al., 2009c; Toepfer et al., 2009; Lundgren et al., 2010), and that habitat alterations to encourage this form of mortality may help reduce pest populations below economic levels. To this end, Lundgren and Fergen (2010) incorporated winter vegetation (i.e., a winter cover crop) into agroecosystems prior to planting maize and observed increases in predator abundance, decreases in pest abundance, and reductions in root damage to the crop. Here, we employ qPCR-based gut content analysis and predation on sentinel pests to test whether (1) winter vegetation increases predation on the pest, and (2) predation on the pest reduces crop damage. Additionally, we (3) establish the relative intensities of interactions between predators and life stages of *D. virgifera*.

2. Methods

2.1. Treatment establishment and sampling procedures

Research was conducted during 2007 and 2008 near Brookings, SD, USA (latitude, longitude: 44.348, −96.811). A 12.5-ha no-till field was divided evenly into annually rotated corn and soybean halves. Maize (glyphosate-tolerant DeKalb 44–92; Monsanto Company, St. Louis, MO, USA) was planted at 77,000 plants ha^{-1} (76 cm between rows) in late May. The maize was fertilized with 169 kg N ha^{-1} prior to planting, and glyphosate was applied at 3.3 L ha^{-1} (Roundup Weathermax, Monsanto Company) prior to planting. Experimental plots (18 m × 24 m each; $n = 6$, 8 in 2007, 2008, respectively) were established into the soybean half of the field in the years prior to the experiments. A randomly and evenly assigned set of the plots was fall-planted in early September with slender wheatgrass, *Elymus trachycaulus* (Link) Gould ex Shinners (Poaceae) (cv. Revenue, Milborn Seeds, Brookings, SD, USA), for use as a winter cover crop (broadcasted at 34 kg ha^{-1}) (Osborne et al., 2008). The cover crop was killed with glyphosate before planting maize, leaving only the residue behind. The remaining plots were maintained as bare soil with glyphosate. Mowed grass alleyways (6–12 m wide) separated plots.

Twenty-five days prior to planting maize, plots were infested with *D. virgifera* eggs that were produced at NCARL, USDA-ARS in Brookings (protocols discussed by Sutter and Branson, 1986). Specifically, 3000 and 3300 viable eggs m^{-1} in 2007 and 2008, respectively, were placed in the maize row using a tractor-mounted egg infester. Resultant larval populations of *D. virgifera* were sampled using weekly soil core samples (10 cm diam., 10 cm deep), collected from the soil at the bases of 10 plants $plot^{-1}$ $date^{-1}$ (four sample dates in 2007 and ten sample dates in 2008). Larvae were extracted from the soil over 7 days into 70% ethanol using Berlese funnels, and 1st, 2nd, or 3rd instars were distinguished based on their head capsule widths. Adult populations were collected weekly in emergence cages (0.61 m × 0.76 m, $n = 5$ $plot^{-1}$), which were evenly spaced along a centralized linear transect through each plot soon after when 3rd instars were detected. Herbivore damage to the roots of 15 plants per plot were assessed destructively using the 1–6 Iowa rating scale (Hills and Peters, 1971). Additional details on these experimental procedures, and the abundance and diversity of insect communities in the two treatments is published in Lundgren and Fergen (2010).

2.2. Predator collection

Predator populations were hand-collected from the soil surface (both years), and extracted from the soil column (2008 only). In both years, predators were hand-collected from quadrat samples ($n = 3$ $plot^{-1}$) beginning at approximately 09:00 on six dates between 18-May and 5-July. In 2008, quadrat samples were collected on seven dates between 21-May and 18-July (see Fig. 1 for 2007 and 2008 sample dates). For each sample, a 0.5-m square, sheet-metal quadrat (15 cm tall) was pressed into the soil at a randomly selected site (e.g., Lundgren et al., 2006). Predators within the quadrat were aspirated by mouth into vials, and were frozen at −20 °C in 70% ethanol until processing. In 2008, predators that emerged from the Berlese funnels (used for sampling pest larvae) within 24 h of collection were placed in 70% ethanol and stored at −20 °C.

2.3. Gut content analysis

Predators that consumed *D. virgifera* in the field were identified using qPCR-based gut content analysis. In 2007, all predators collected from the soil surface were analyzed (536 specimens). In 2008, the qPCR resources were split between surface- and soil column-captured predators (432 and 384 specimens, respectively). Approximately seven surface-collected predators were randomly selected from each plot on each sample date in 2008. For the predators collected in the soil column, we randomly selected approximately five predators from each treatment on each sample date. Prior to analysis, each specimen was identified to as fine a

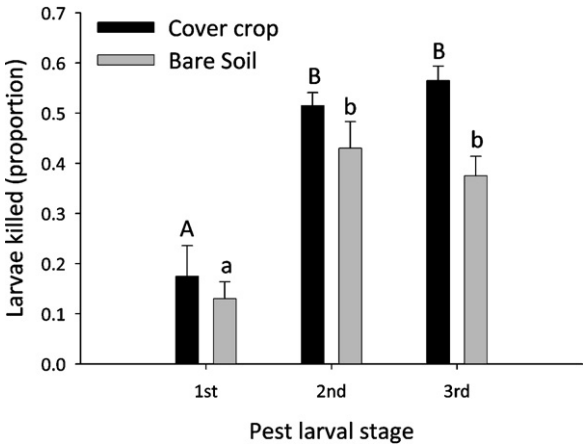

Fig. 1. Mean (SEM) predation rates on restrained *Diabrotica virgifera* larvae in cover-cropped and bare soil. Restrained larvae were 3rd instars, placed in the fields ($n = 4$ plots per treatment) when natural pest populations were in the 1st, 2nd, and 3rd stadia. There were significantly higher predation rates in the cover cropped plots than in the bare soil treatments (see text for statistics). Within a treatment, pairwise Kruskal Wallis non-parametric ANOVAs were used to determine differences in predation rates during the three pest life stages. Bars within a treatment topped with different letters (uppercase letters refer to cover crop and lowercase refer to bare soil) are significantly different ($\alpha = 0.05$).

taxonomic grouping as possible, and voucher specimens were kept of each morpho-type for later identification (identities and numbers of specimens analyzed are presented in Appendices 1 & 2). DNA was extracted from only the digestive tracts of predators longer than 1 cm to reduce tissue clogging the extraction filters.

Individual predators were analyzed for the presence of *D. virgifera* DNA. DNA was extracted using DNeasy® blood and tissue extraction kits (Qiagen, Valencia, CA, USA) and stored according to product instructions. All tissues were macerated in ATL buffer with autoclaved pestels and incubated with proteinase K for 3 h. A previously published primer set (Lundgren et al., 2009c) that is specific to *D. virgifera* was used in subsequent reactions. These primers (forward: 5′-TAGTTCCCTTAATAATTGCTGCTC-3′; reverse: 5′-CCCCCTTTCTACTATCCTTCTTA-3′) amplify a 119-bp sequence of the COI and tRNA-Leu genes, and >100 non-target species living within maize fields have been screened for negative results (Lundgren et al., 2009c). None of these non-target specimens were amplified by the primer sets under the specified reaction conditions.

The DNA of each predator was amplified in 25-μL PCR reactions. Components of the reaction were 12.5 μL 2× Brilliant SYBR Green qPCR master mix (Qiagen), 225 nmol/L of each primer, 1 μL template DNA, and 9.5 μL of PCR-water. Reactions were run on an MX3000P qPCR machine (Stratagene, La Jolla, CA, USA) using the following conditions: 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 normalization of the reactions) during the annealing step of each PCR cycle. Fluorescence was adjusted manually to bring the baseline-corrected normalized fluorescence (dRn) just above background fluorescence for each plate. On each 96-well plate, a series of five positive controls from a single extraction of *D. virgifera*, and three no-template controls were included. Following the PCR reaction, the products were melted to determine their dissociation temperature; samples were heated to 95 °C, then cooled to 55 °C and the temperature was ramped to 95 °C at a rate of 0.2 °C/s while monitoring fluorescence continuously. In qPCR, the melt temperature of a PCR product is a simple, free, and less subjective surrogate for the electrophoretic gels typically conducted after conventional PCR to verify the presence of the targeted, species-specific DNA sequence.

For each positive sample, the Ct (or PCR cycle at which fluorescence is detected above background) was recorded. This Ct is negatively correlated with the amount of target DNA in the sample. Three parameters were derived from these gut content analyses as metrics of predation intensity per plot: relative of detection (or proportion positive for *D. virgifera* DNA), $Ct^{-1} \times 100$ (i.e., DNA quantity), and predation index (number of specimens collected per plot × [frequency of predation per plot] × [mean DNA quantity per plot]). These data were calculated for each predator taxon. It is important to note that frequency of predation and the predation index calculated here are both affected by the prey size, and we might expect to observe higher detection levels as the prey species grows in size (Weber and Lundgren, 2009b).

2.4. Predation on restrained larvae

During 2008, predation intensity on restrained sentinel *D. virgifera* larvae was used as an additional metric of predation during the 1st (20-June), 2nd (1-July), and 3rd (15-July) stadia of the field populations of pests (as revealed by core samples). Larvae of *D. virgifera* were reared to the 3rd stadium on maize roots (Branson et al., 1975). One hour prior to field observations, individual larvae were pierced through their posterior segments with a size-0 insect pin; the pins and larvae were then fastened to 1-cm clay balls (Frank and Shrewsbury, 2004; Lundgren et al., 2006). Larvae can survive for several hours while restrained under these conditions.

A 5 × 5 centralized grid of 25 observation sites were established in each plot. For each site, a restrained larva was placed flush with the soil surface at the base of a maize plant. After 1 h, the larva's status at each observation site was noted (alive, missing or dead), and any predators attacking the larva were identified. Predation observations were conducted at both 09:30 and 22:00 on each sample date to partially capture the distinct diurnal and nocturnal predator communities that inhabit maize fields (Brust et al., 1986; Lundgren et al., 2009b, 2010). The percent of larvae killed (either missing or dead) per plot was calculated for each sample date, with data over the two diel sample periods being pooled.

2.5. Data analysis

All proportional data were transformed to the arcsine of the square-root of the mean prior to analysis. The proportions of restrained *D. virgifera* larvae per plot killed during the three pest instars were compared between the cover cropped and bare soil treatments using a repeated-measures ANOVA (treatment was the main factor, and the three sample dates were the within-subject parameters). The mean proportions of predators positive for *D. virgifera* DNA, mean quantities of DNA detected per predator ($Ct^{-1} \times 100$), and mean predation indices per plot were compared between cover-cropped and bare soil treatments using three independent ANOVAs.

Proportions of restrained larvae killed per plot (pooled over the three sample dates and diel periods) were related with mean root damage per plot, adult populations per plot, and total larvae per plot using independent simple regression analyses. The proportions of restrained larvae killed per plot during each stadium of the pest were related with the corresponding number of *D. virgifera* 1st, 2nd, and 3rd instars collected per plot using independent simple regression analyses. The proportion of specimens testing positive for *D. virgifera* DNA per plot, the mean Cts per plot, and the mean predation indices per plot were related with the numbers of 1st, 2nd and 3rd instars, total larvae and adults captured and the mean root damage observed per plot using independent regression analyses.

Table 1
Predator groups recovered from the soil surface and their predation on *Diabrotica virgifera* as measured by qPCR. The mean ± SEM numbers of specimens collected, frequencies of detection and quantity of *D. virgifera* DNA detected per insect per plot are presented. Predators with asterisks have sucking mouthparts.

Order	Family	Total specimens collected (total across plots)	Total specimens analyzed	Relative frequency of detection (n plots)	Quantity of DNA; $100 \times Ct^{-1}$	Predation index[a]
Araneae	*	2.5 ± 1.13 (35)	16	0 (6)		
Araneae	Anyphaenidae*	0.57 ± 0.23 (8)	4	0 (4)		
Araneae	Araneidae*	2.93 ± 0.87 (41)	28	0.10 ± 0.10 (10)	0.24 ± 0.24	0.24 ± 0.24
Araneae	Clubionidae*	0.21 ± 0.15 (3)	2	0.5 (1)	2.38	2.38
Araneae	Dictynidae*	0.79 ± 0.19 (11)	9	0 (8)		
Araneae	Gnaphosidae*	1.36 ± 0.25 (19)	16	0.30 ± 0.15 (10)	0.83 ± 0.42	1.10 ± 0.61
Araneae	Linyphiidae*	17.93 ± 3.65 (251)	174	0.05 ± 0.02 (14)	1.30 ± 0.36	2.22 ± 0.94
Araneae	Liocranidae*	0.07 ± 0.07 (1)	1	0 (1)		
Araneae	Lycosidae*	1.43 ± 0.40 (20)	14	0.32 ± 0.13 (7)	1.11 ± 0.53	1.93 ± 1.20
Araneae	Oecobiidae*	0.43 ± 0.25 (6)	4	0 (3)		
Araneae	Philodromidae*	1.79 ± 0.54 (25)	6	0.20 ± 0.20 (5)	0.49 ± 0.49	0.49 ± 0.49
Araneae	Pisauridae*	0.43 ± 0.25 (6)	4	0 (3)		
Araneae	Salticidae*	1.43 ± 0.40 (20)	18	0.11 ± 0.11 (9)	0.32 ± 0.32	0.32 ± 0.32
Araneae	Tetragnathidae*	4.36 ± 1.20 (61)	58	0.02 ± 0.01 (10)	0.64 ± 0.45	0.65 ± 0.47
Araneae	Thomisidae*	1.07 ± 0.27 (15)	11	0 (6)		
Chilopoda	*	2.71 ± 0.42 (38)	30	0.21 ± 0.10 (11)	0.96 ± 0.41	1.40 ± 0.62
Coleoptera	Carabidae (larvae)*	1.21 ± 0.32 (17)	7	0 (3)		
Coleoptera	Carabidae (adults)	13.64 ± 1.73 (191)	128	0.10 ± 0.03 (14)	1.53 ± 0.37	4.98 ± 1.83
Coleoptera	Coccinellidae	27.21 ± 11.70 (381)	59	0.03 ± 0.02 (8)	0.68 ± 0.45	4.57 ± 4.19
Coleoptera	Lampyridae	0.07 ± 0.07 (1)	1	0 (1)		
Coleoptera	Staphylinidae	3.86 ± 1.06 (54)	36	0.03 ± 0.03 (10)	0.37 ± 0.37	0.37 ± 0.37
Hemiptera	Geocoridae*	3.71 ± 0.95 (52)	26	0.17 ± 0.14 (7)	0.68 ± 0.44	0.75 ± 0.48
Hemiptera	Miridae*	0.14 ± 0.14 (2)	1	0 (1)		
Hymenoptera	Formicidae*	10.64 ± 2.05 (149)	84	0.11 ± 0.03 (13)	1.38 ± 0.37	2.68 ± 0.90
Opiliones	Phalangiidae	3.86 ± 0.64 (54)	44	0.06 ± 0.04 (13)	0.41 ± 0.28	0.85 ± 0.61
Orthoptera	Gryllidae	22.64 ± 4.01 (317)	169	0.03 ± 0.02 (14)	0.50 ± 0.26	0.87 ± 0.48

[a] Predation index for each family equals $N \times 100 \times$ relative frequency of detection per plot $\times Ct^{-1}$ per plot.

All analyses were conducted using Systat 11 Software (Richmond, CA, USA).

3. Results

3.1. Identification of D. virgifera predators

3.1.1. Predators of restrained larvae

On average (±SEM), 15.3 ± 3.10, 47.5 ± 2.63, and 47.0 ± 5.31% of sentinel larvae per plot were killed during 1 h of exposure during the 1st, 2nd, and 3rd stadia of natural pest populations. Predators observed eating were crickets (*Allonemobius* sp.; Orthoptera: Gryllidae) ($n = 48$ observed events), harvestmen (*Phalangium opilio*; Opiliones: Phalangiidae) (10), an ant species (Hymenoptera: Formicidae) (4), *Pterostichus permundus* (Coleoptera: Carabidae) (3), big-eyed bug nymphs (*Geocoris* sp.; Hemiptera: Geocoridae) (2), two spiders (Araneae) (2 and 2), *Coleomegilla maculata* larva (Coleoptera: Coccinellidae) (1), *Cyclotrachelus alternans* (Coleoptera: Carabidae) (1), a wolf spider (Araneae: Lycosidae) (1), *Poecilus chalcites* (Coleoptera: Carabidae) (1), and *Poecilus lucublandus* (Coleoptera: Carabidae) (1).

3.1.2. Gut content analysis (soil surface)

Of the 79 predator taxa (950 specimens) analyzed from the soil surface (1950 total were collected over 2 year), 33 taxa from seven orders tested positive for *D. virgifera* DNA. Linyphiidae (18.3% of specimens), crickets (17.8%), and Carabidae adults (13.5%) were the most frequently collected predator taxa collected on the soil surface (Table 1; Appendix 1). The maximum relative frequency of detection within any single taxon was 32% (Lycosidae; Table 1). Carabidae adults (1.53), Formicidae (1.38), and Linyphiidae (1.30) had the most *D. virgifera* DNA detectable in their guts (i.e., highest $Ct^{-1} \times 100$) (Table 1). Carabidae adults and Coccinellidae adults (driven largely by *Scymnus* adults), Formicidae and Linyphiidae had the highest predation indices; all other taxa had predation indices lower than 2.00 (Table 1). Ten taxa collected from the soil surface did not consume *D. virgifera*, although sample sizes for these taxa were notably small (Table 1).

3.1.3. Gut content analysis (soil column)

Of the 30 predator taxa (384 specimens) analyzed from the soil column (1967 predators were collected), *D. virgifera* DNA was detected in 20 predator taxa from seven families (five orders) (Table 2; Appendix 2). Japygidae, Staphylinidae, Carabidae, and Formicidae dominated the predator community in the soil cores, accounting for 85% of specimens collected. None of the predator families had more than 26% of specimens testing positive for the herbivore's DNA. Carabidae adults and Formicidae had the most *D. virgifera* DNA (Table 2). Japygidae, Formicidae, and Carabidae adults had a substantially higher predation index than the other taxa (Carabidae larvae also had a high predation index) (Table 2). Spiderlings and Gryllidae from the soil column did not feed on *D. virgifera*, although the sample size for Gryllidae was quite low. Predators collected from Berlese funnels with *D. virgifera* larvae were not artificially contaminated with target DNA subsequent to field sampling. Core samples containing *D. virgifera* larvae did not accompany a greater relative frequency of detection, Ct, or predation index in predators from the same core sample (frequency: $F_{1,78} = 0.73$, $P = 0.40$; Ct: $F_{1,78} = 1.09$, $P = 0.30$; predation index: $F_{1,78} = 1.15$, $P = 0.29$).

3.2. The effects of winter vegetation on predation

3.2.1. Predation on restrained larvae

Predation was higher on the sentinels in the plots with cover crop residue than in the bare soil plots, and predation increased as the season progressed (treatment: $F_{1,6} = 9.93$, $P = 0.02$; time: $F_{1,2} = 36.64$, $P < 0.0001$; time × treatment: $F_{2,12} = 1.52$, $P = 0.26$) (Fig. 1). In both the cover cropped and bare soil treatments, predation increased during later in the season (Fig. 1).

Table 2
Predator groups recovered from the soil column and their predation on *Diabrotica virgifera* as measured by qPCR. The mean ± SEM numbers collected frequencies of detection and quantity of *D. virgifera* DNA detected per insect per plot are presented. Predators with asterisks have sucking mouthparts.

Order	Family	Total collected (total across plots)	Number analyzed	Relative frequency of detection (n plots)	Quantity of DNA; $100 \times Ct^{-1}$	Predation index[a]
Araneae	Spiderlings*	28.50 ± 5.78 (228)	9	0		
Araneae	Linyphiidae*	4.38 ± 1.22 (35)	15	0.15 ± 0.10 (6)	0.87 ± 0.56	2.52 ± 2.09
Chilopoda		10.38 ± 1.71 (83)	13	0.19 ± 0.09 (7)	1.10 ± 0.52	5.43 ± 2.95
Coleoptera	Carabidae (larvae)*	20.50 ± 2.86 (164)	19	0.25 ± 0.14 (7)	1.20 ± 0.57	14.77 ± 10.54
Coleoptera	Carabidae (adults)	26.00 ± 3.46 (209)	60	0.26 ± 0.08 (8)	2.24 ± 0.50	18.99 ± 6.05
Coleoptera	Staphylinidae	27.75 ± 3.10 (222)	75	0.15 ± 0.05 (8)	2.02 ± 0.45	10.34 ± 2.92
Diplura	Japygidae	77.88 ± 4.73 (623)	106	0.13 ± 0.05 (8)	1.99 ± 0.44	26.41 ± 9.83
Hymenoptera	Formicidae*	37.75 ± 9.21 (302)	81	0.21 ± 0.05 (8)	2.40 ± 0.03	25.23 ± 9.70
Orthoptera	Gryllidae	3.75 ± 1.46 (30)	3	0 (3)		

[a] Predation index for each family equals *N* specimens collected per plot × 100 × relative frequency of detection per plot × Ct^{-1} per plot.

3.2.2. Gut content analyses

There were no treatment effects of winter cover crop residue on the relative frequency of predation detection per plot by predators from the soil surface ($F_{1,12} = 1.49$, $P = 0.25$), mean quantity of DNA detected in the predators (i.e., $Ct^{-1} \times 100$; $F_{1,12} = 0.89$, $P = 0.36$), or the mean predation index calculated per predator per plot ($F_{1,12} = 2.00$, $P = 0.18$). For predators collected at the soil surface, mean (SEM) detection frequencies were 5.39 ± 1.69 and 7.56 ± 1.18% of predators per plot, quantities of DNA detected per plot were 2.25 ± 0.38 and 2.62 ± 0.06 ($Ct^{-1} \times 100$), and predation indices were 35.7 ± 15.4 and 13.6 ± 2.45 in the cover cropped and bare soil treatments, respectively. A similar lack of treatment effects were observed in the gut content analyses performed on predators collected from the soil column (frequency of detection: $F_{1,6} = 2.54$, $P = 0.16$; $Ct^{-1} \times 100$: $F_{1,6} = 0.30$, $P = 0.61$; predation index: $F_{1,6} = 0.62$, $P = 0.46$). For predators collected from the soil column, mean (SEM) frequencies of detection were 14.3 ± 4.84 and 21.8 ± 1.81% of predators per plot, quantities of DNA detected per plot were 2.71 ± 0.09 and 2.76 ± 0.06 ($Ct^{-1} \times 100$), and predation indices were 103 ± 34.4 and 134 ± 21.1 in the cover cropped and bare soil treatments, respectively.

3.3. Predation metrics and levels of pest suppression

3.3.1. Predation on restrained larvae

Predation rates during the 3rd stadium of the pest were strongly and inversely correlated with pest densities ($F_{1,6} = 14.59$, $P = 0.009$) (Fig. 2A). Predation rates observed during the 1st and 2nd stadia of the pest were not correlated with the numbers of 1st and 2nd instars collected (1st instars: $F_{1,6} = 0.71$, $P = 0.43$; 2nd instars: $F_{1,6} = 0.01$, $P = 0.91$). Likewise, season-long predation levels per plot were uncorrelated with total larval densities, nor with total adults collected per plot (larvae: $F_{1,6} = 0.04$, $P = 0.85$; adults: $F_{1,6} = 0.27$, $P = 0.62$). The proportion of larvae killed per plot was strongly and inversely correlated with the root damage experienced in each plot ($F_{1,6} = 7.44$, $P = 0.03$) (Fig. 3A).

3.3.2. Gut content analyses

With a few important exceptions, metrics from gut content analyses (relative frequency of detection, mean pest DNA quantity, and mean predation index) were not correlated with the total larvae or adults collected in each plot, nor with the root damage experienced. This was true for predators collected on the soil surface and in the soil column (Table 3). Exceptions were that the Predation Index of predators collected on the soil surface per plot was strongly and negatively correlated with root damage per plot (Fig. 3B) and the densities of 3rd instars per plot (Fig. 2B). Also, the Ct^{-1} of predators in the soil column was negatively correlated with the abundance of 2nd instars of *D. virgifera*.

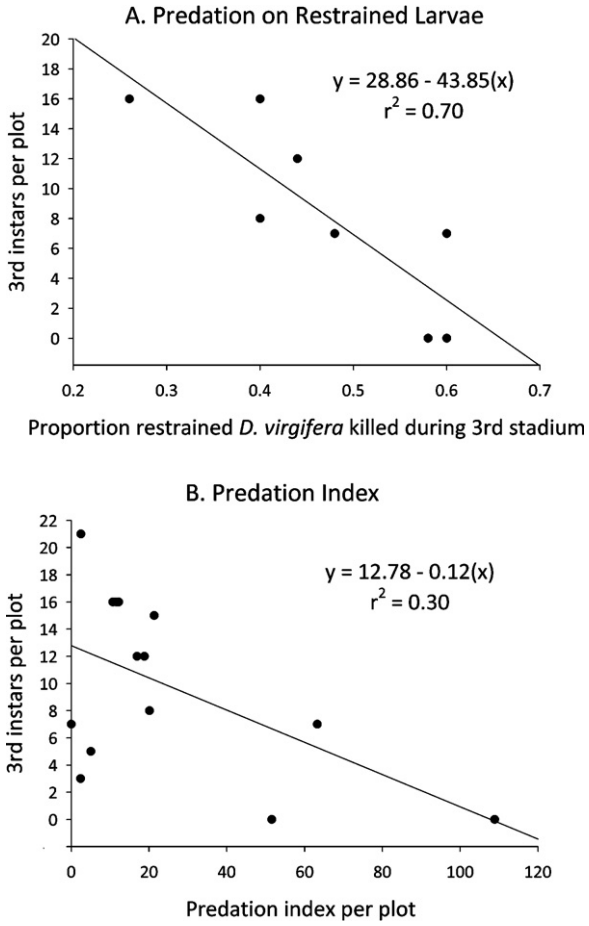

Fig. 2. Relationship between predation levels on restrained larvae of *Diabrotica virgifera* and the number of *D. virgifera* 3rd instars recovered per plot in corn fields. (A) Predation rates assessed using sentinel larvae. (B) Predation index resulting from the qPCR-based gut content analysis (N specimens collected per plot × 100 × relative frequency of detection per plot × Ct^{-1} per plot). Each datapoint represents a single experimental plot.

To summarize, predation metrics generated by PCR-based gut content analyses (on surface active predators) and observations on restrained larvae confirmed the inverse relationship between predation intensity and 3rd instars and root damage estimates. Moreover, these 3rd instar densities per plot were positively correlated with plant damage levels ($F_{1,12} = 20.55$, $P = 0.001$; $r^2 = 0.63$; slope = 0.13), supporting our hypothesis that the pest is causing the observed root damage. Additionally, the quantity of DNA found in the stomachs of predators from the soil column was inversely correlated with densities of 2nd instar pests.

Table 3
Gut content analysis of predators were used to identify consumption of *Diabrotica virgifera*, and the results of various regression analyses between parameters (relative frequencies of predation, DNA quantity,[a] and the predation index[b]) derived from these gut content analyses and *D. virgifera* and corn crop characteristics observed are presented. The life stages of the pest are included as columns, as is a measure of root damage inflicted by *D. virgifera*.

Predation parameter	1st instars	2nd instars	3rd instars	Total larvae	Total adults	Root ratings
Surface active predators						
Relative frequency of detection	$F_{1,12}$ = 0.03; P = 0.87; slope = −11.59	$F_{1,2}$ = 0.02; P = 0.86; slope = −2.71	$F_{1,12}$ = 0.01; P = 0.92; slope = 1.77	$F_{1,12}$ = 0.02; P = 0.88; slope = −10.59	$F_{1,12}$ = 0.04; P = 0.85; slope = −7.04	$F_{1,12}$ = 0.14; P = 0.72; slope = 1.06
Ct^{-1}	$F_{1,12}$ = 0.06; P = 0.81; slope = −245.54	$F_{1,12}$ = 0.14; P = 0.71; slope = 83.59	$F_{1,12}$ = 0.13; P = 0.72; slope = 94.28	$F_{1,12}$ = 0.002; P = 0.97; slope = −42.04	$F_{1,12}$ = 0.09; P = 0.77; slope = 158.73	$F_{1,12}$ = 1.97; P = 0.18; slope = 55.53
Predation index	$F_{1,12}$ = 0.05; P = 0.83; slope = −0.05	$F_{1,12}$ = 0.88; P = 0.37; slope = −0.05	$F_{1,12}$ = 5.16; P = 0.04; slope = −0.12	$F_{1,12}$ = 0.90; P = 0.36; slope = −0.22	$F_{1,12}$ = 1.09; P = 0.32; slope = −0.13	$F_{1,12}$ = 7.08; P = 0.02; slope = −0.02
Predators from soil column						
Relative frequency of detection	$F_{1,6}$ = 1.82; P = 0.23; slope = −94.49	$F_{1,6}$ = 1.96; P = 0.21; slope = −12.63	$F_{1,6}$ = 0.27; P = 0.62; slope = 11.96	$F_{1,6}$ = 1.17; P = 0.32; slope = −92.60	$F_{1,6}$ = 1.24; P = 0.31; slope = −39.31	$F_{1,6}$ = 0.44; P = 0.53; slope = 2.37
Ct^{-1}	$F_{1,6}$ = 0.69; P = 0.44; slope = 4763.63	$F_{1,6}$ = 6.04; P = 0.049; slope = −12.63	$F_{1,6}$ = 0.17; P = 0.70; slope = 11.96	$F_{1,6}$ = 0.69; P = 0.44; slope = 5537.08	$F_{1,6}$ = 0.89; P = 0.38; slope = −2582.10	$F_{1,6}$ = 0.40; P = 0.55; slope = 170.32
Predation index	$F_{1,6}$ = 1.26; P = 0.30; slope = −0.16	$F_{1,6}$ = 2.28; P = 0.19; slope = −0.03	$F_{1,6}$ = 0.00; P = 0.99; slope = −7.81	$F_{1,6}$ = 1.24; P = 0.31; slope = −92.60	$F_{1,6}$ = 0.92; P = 0.38; slope = −0.07	$F_{1,6}$ = 0.05; P = 0.83; slope = 1.59

[a] The quantity of DNA is measured as the Ct, or cycle threshold, and is inversely related to DNA quantity.
[b] Predation index for each family equals N specimens collected per plot × 100 × relative frequency of detection per plot × Ct^{-1} per plot.

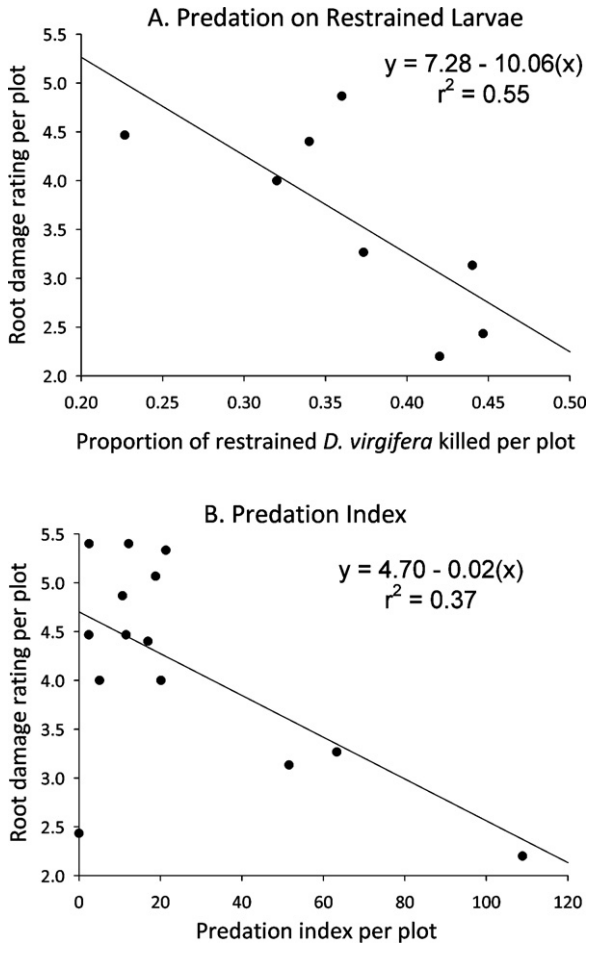

Fig. 3. Relationship between predation levels on restrained *Diabrotica virgifera* larvae and pest-derived plant damage. (A) Predation rates assessed on restrained larvae. (B) Predation index resulting from the qPCR-based gut content analysis (N specimens collected per plot × 100 × relative frequency of detection per plot × Ct^{-1} per plot). Each datapoint represents a single experimental plot. Root ratings follow the Iowa scale (1–6; lower ratings correlate with less damage), and season-long predation rates are pooled across diurnal observation periods for each plot.

4. Discussion

A diverse soil predator community consumes many agricultural pests under field conditions, and farm management practices can conserve predator communities within cropland and increase their impact on pest populations and their damage. Previous work shows that cover crops reduce pests in cropland, but very few have identified mechanisms for how this happens. The current study shows that winter vegetation, or its resulting residue, increases predation rates on the key herbivore of North American maize production systems. Moreover, increasing predation rates in a cornfield are strongly correlated with reductions in the larval densities of *D. virgifera* and the damage inflicted onto the crop plant.

In this system, gut content analysis and the predation intensity on restrained larvae indicate that predation is particularly intense during the third stadium of the herbivore. Previously, we showed that 3rd instars of *D. virgifera* were significantly reduced in cover-cropped maize fields (Lundgren and Fergen, 2010), and we hypothesized that predation was largely responsible for this phenomenon. *Diabrotica virgifera* larvae reside within corn roots and thereby likely avoid foraging predators. However, larvae of *D. virgifera* leave their refuges in search of higher quality host roots and to find pupation sites (Branson et al., 1975; Strnad and Bergman, 1987; Hibbard et al., 2004). Moreover, they have evolved a potent anti-predator hemolymph defense that is most effective during the 3rd stadium (Lundgren et al., 2009a, 2010), presumably to protect them from predation when they leave the roots. We hypothesize that cover crop residue increases predation intensity on 3rd instars of the pest by concurrently (1) providing better habitat for predators so that densities are relatively high (Lundgren and Fergen, 2010), and (2) affecting the physiology of maize roots, requiring the 3rd instars to leave the plant earlier in order to find more suitable host roots, thereby exposing them to this aggregated predator community. Additional experiments are underway to determine the effects of cover crops on host suitability of the crop.

A number of factors restrict which predator species within a community rely on a given prey item, including the spatio-temporal correlations among the species, the nutritional status of the predator, and the defenses of the prey. The gut content analysis from the soil column and the surface active communities agreed in their assignment of a high predation index to Carabidae adults (carabid beetles) and Formicidae (ants); Japygidae and *Scymnus* (a lady

beetle; Coccinellidae) also had notably high predation indices in the soil column and surface-active communities, respectively. This current study builds upon previous work on this system (Lundgren et al., 2009c) by incorporating actual densities of the predator community in the soil column and surface-dwelling communities into the predation index to more accurately portray the relative contributions of predator taxa to *D. virgifera* predation. Previous gut content analysis work on maize food webs revealed a particularly strong trophic linkage between predators with sucking mouthparts and the pest (the top predation indices were given to predator families with sucking mouthparts; Tables 1 and 2). Previous work has shown that sucking predators are less prone to the *D. virgifera*'s hemolymph defense (Lundgren et al., 2009a,c, 2010); the current research is consistent with this pattern. It is important to note that no single predator species dominated the community, and the dynamic nature of generalist predator communities precluded our ability to target conservation efforts on any specific predator. We advocate the conservation of predator communities as a whole within agroecosystems in order to provide the plasticity that will allow them to adapt to the changing target pest population (Cardinale et al., 2006; Straub and Snyder, 2008).

This study illustrates that multiple techniques and approaches reveal a more complete story of how generalist predators interact with other trophic levels compared to using a single predation metric. qPCR-based gut content analysis was an excellent method for identifying the breadth of species that consumed *D. virgifera* immatures under field conditions. But these gut content analyses results were inconsistent in their portrayal of the interactions between the pest and natural enemy complex. Predation measured with gut analysis was roughly three times more frequent in the soil column than at the soil surface, but the former was uncorrelated with prey population dynamics or their impact on the pest. In the surface-active predator community, although the predation index was nearly three times greater in the cover cropped plots than in the bare soil plots, the variability in gut content analysis led to such low statistical power that this difference was not statistically significant. But it is notable that predation indices of surface active predator communities were inversely related to both the third instar populations of the pest and the root damage inflicted to the crop, a fact that was reconfirmed using predation rates on restrained prey. Predation on restrained pest larvae (sentinels) did not identify the diversity of predators that were consuming *D. virgifera* under natural conditions, but this metric was well correlated with overall predation intensity underway in a given plot, and sentinel larvae were predictive of the impact that predators have on the pest and crop damage. The caveats to interpreting different measures of predation (especially gut content analysis) (Sunderland, 1996) has received much attention; our research suggests that each of these methods has relative strengths, and that the best concept of the trophic interactions in this subterranean food web will be produced by using more than one approach. Moreover, having confirmatory results from both gut content analysis and predation on sentinel prey strongly suggests that third instars of the pest are particularly susceptible to predation, and that the surface-dwelling community is more responsive to the cover crop treatment and pest densities than the soil-dwelling predator community.

Finally, our research shows that native predator communities can reduce pest populations, but for biological control to be practical, we must conserve biodiversity and reduce disturbance in our agroecosystems at both local and landscape scales (Tscharntke et al., 2007; Finke and Snyder, 2008; Macfadyen and Bohan, 2010). A next step in this process will be to determine the economic benefits associated with conservation biological control relative to other approaches to pest management (Cullen et al., 2008). The current study helps to document that incorporating winter cover crops not only increases predator abundance, but also improves their function against *D. virgifera* to the benefit of the crop.

Acknowledgements

We thank Carson Dinger, Mallory Johnson, and John Anderson for assistance with data collection, Michael Seagraves for statistical assistance. Tom Unruh and Don Weber provided helpful comments on previous drafts. Mention of any proprietary products does not constitute endorsement by the USDA.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.apsoil.2011.08.005.

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