

Pest control experiments show benefits of complexity at landscape and local scales

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Abstract. Farms benefit from pest control services provided by nature, but management of these services requires an understanding of how habitat complexity within and around the farm impacts the relationship between agricultural pests and their enemies. Using cage experiments, this study measures the effect of habitat complexity across scales on pest suppression of the cabbage aphid *Brevicoryne brassicae* in broccoli. Our results reveal that proportional reduction of pest density increases with complexity both at the landscape scale (measured by natural habitat cover in the 1 km around the farm) and at the local scale (plant diversity). While high local complexity can compensate for low complexity at landscape scales and vice versa, a delay in natural enemy arrival to locally complex sites in simple landscapes may compromise the enemies' ability to provide adequate control. Local complexity in simplified landscapes may only provide adequate top-down pest control in cooler microclimates with relatively low aphid colonization rates. Even so, strong natural enemy function can be overwhelmed by high rates of pest reproduction or colonization from nearby source habitat.

Key words: *agroecosystems; biological control; Brevicoryne brassicae; cabbage aphid; ecosystem services; natural habitat; pest management; Salinas Valley, California, USA.*

INTRODUCTION

Natural enemies, the predators and parasitoids of agricultural pests, provide a sustainable and efficient alternative to pesticides in some circumstances (e.g., rice, Settle et al. [1996]; cotton and citrus, Ruttan [1999]), but fail strikingly to control pests under other circumstances (Stiling 1993, Collier and Van Steenwyk 2004). Using natural enemies instead of pesticides to control crop pests could provide significant societal benefits by reducing economic, environmental, and public health costs (Pimentel et al. 1992, Eskenazi et al. 2007). To utilize natural pest control services reliably, however, it is critical to understand the conditions under which top-down control can and cannot maintain pest populations below desired levels. This study investigates landscape- and local-scale environmental factors that constrain or enhance the potential contribution of natural enemies in the management of crop pests.

Both local- and landscape-scale factors are thought to contribute to the control of pests by natural enemies. While conservation biological control aims to maximize pest control services by creating or enhancing habitat at the local scale for native or “naturalized” natural

enemies (Fiedler et al. 2008), scientists now recognize the importance of the larger landscape scale in maintaining natural enemy communities (Tscharntke et al. 2005). Structurally complex landscapes, defined as landscapes with high proportions of natural or unmanaged habitat, are associated with increased abundance and diversity of natural enemies on farms, across many different geographic locations and crop types (Bianchi et al. 2006, Chaplin-Kramer et al. 2011b). The resources provided for natural enemies in complex landscapes may complement or substitute for local diversity in agroecosystems (Tscharntke et al. 2005). The reverse may not hold true, however. It has been suggested that local-scale habitat improvements may not support a viable natural enemy community on their own, but only serve to concentrate enemies already supported by natural habitat in the landscape surrounding the farm (Gurr et al. 1998). Both scales are likely important to the maintenance of healthy enemy communities, however, and the interplay between them is not well understood. Some studies have investigated the effects of complexity at local and landscape scales simultaneously, but it has proven difficult to resolve the relative contribution of each factor (Elliott et al. 1998, Thies and Tscharntke 1999, Letourneau and Goldstein 2001, Östman et al. 2001, Clough et al. 2005, Bianchi et al. 2008, Werling and Gratton 2008). Local and landscape complexity are also often correlated; small diverse farms tend to be found in small wooded valleys and large monocultures are more likely to be in vast agricultural areas.

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An additional difficulty in understanding the relationship between pest control services and complexity at any scale is that both direct and indirect effects can be operating and may counteract one another. Habitat complexity at either scale may benefit crop pests directly if it provides alternate host plants or predator refuges (Landis et al. 2000, Chaplin-Kramer et al. 2011a), or it may constrain pests directly if it limits dispersal into or across agricultural fields (den Belder et al. 2002). Habitat complexity may also indirectly constrain pests through enhancement of natural enemy communities that provide top-down control (Tscharntke et al. 2005). One step in understanding how habitat complexity contributes to overall pest management is to determine how it influences natural enemy populations, which in turn affect pest population dynamics.

The vast majority of studies investigating the effects of complexity on pest control services have stopped short of actually measuring the service, instead using proxies such as natural enemy abundance or predation rate (Letourneau and Bothwell 2008, Chaplin-Kramer et al. 2011b). The first objective of this study is to determine the degree to which natural enemies can reduce pest populations, by measuring pest population growth in the presence and absence of their enemies. The second objective is to determine whether increased natural enemy density or diversity associated with increased habitat complexity confers enhanced pest control services, by employing enemy exclusion experiments across a complexity gradient. While previous studies have utilized cage experiments to demonstrate enhanced pest control services along landscape gradients (Gardiner et al. 2009), no studies have compared the effects of complexity across scales. Therefore, the ultimate objective of this study is to measure differences in pest control services provided by resident natural enemies across a gradient of habitat complexity at two scales. We test the hypotheses that (1) cross-scale habitat complexity (i.e., complexity at both landscape and local scales, compared to complexity at only one scale) provides superior pest control services to farms, and (2) one scale of complexity can substitute for another.

For the purposes of this paper, we draw a distinction between pest control services and pest control. *Pest control* is defined according to a predesignated threshold known as the economic injury level, which has little to do with the ecology or predator-prey dynamics of a system. We define pest control *services* as the ecosystem service that results in a reduction of pest populations from the level they would achieve in the absence of that service. For that reason, we focus more on comparative measures of pest reduction rather than absolute measures such as pest densities. We acknowledge that pest control *services* as defined here may not always result in economic pest *control*, but suggest that better understanding the delivery of these services could be an important step toward achieving more sustainable and reliable pest management (Kremen 2005).

MATERIALS AND METHODS

Study system

The cabbage aphid *Brevicoryne brassicae* (Linnaeus) is a major pest of broccoli. Aphids are able to build up populations quickly following colonization because they can reproduce asexually, giving birth to several live young (nymphs) each day once they reach maturity. There are alate (winged) and apterous (wingless) morphs of adult aphids, with intraspecific competition triggering the production of alate individuals, who then seek out uncolonized plants and may spend several days producing nymphs before moving onto the next plant (Dixon 1977). The most abundant natural enemies of the cabbage aphid in the study region are the larvae of various syrphid flies (Diptera: Syrphidae). Other enemies include the parasitic wasp *Diaeretiella rapae*, coccinellid beetles (Coleoptera: Coccinellidae), the lacewings *Chrysoperla* and *Hemerobius* species, the aphid midge *Aphidoletes aphidimyza*, spiders, and a variety of other coleopteran and hemipteran predators (van Emden 1963). Most of these enemies are extremely mobile and forage in many different habitats for floral resources and/or alternate prey, making floral resources on farms or in the surrounding habitat an important determinant of their distribution in crop fields.

Study sites

The study was conducted in 2008 and 2009 on 10 organic broccoli farms on California's Central Coast, in Santa Cruz, Monterey, and San Benito Counties (Fig. 1). The same sites were repeated across years and seasons to avoid confounding spatial and temporal variation (using the same fields within each farm whenever possible, or the nearest field planted in broccoli if crops were rotated in subsequent years). The only pesticide used on broccoli at these sites was M-PEDE (Dow AgroSciences, Indianapolis, Indiana, USA), a nonpersistent insecticidal soap, which has been shown to have temporary to no effects on natural enemies (UC IPM 2008). At all sites, the crop around the study area was not sprayed during the course of the experiment. Study sites were characterized by the amount of surrounding natural or seminatural habitat, which included riparian habitat, chaparral scrub, deciduous and coniferous woodland, and grasslands that were often degraded and/or invaded by nonnative weeds. Transects along farm field edges at each site recorded the presence or absence of nearby weedy patches of the mustard *Brassica nigra*, which may provide a predator refuge for *Brevicoryne brassicae* (Chaplin-Kramer et al. 2011a).

Sites were selected at either end of a landscape complexity gradient, with the surrounding landscapes composed of predominantly natural habitat (69% \pm 8% natural habitat, 10% \pm 7% agriculture, mean \pm SE) or predominantly agricultural habitat (4% \pm 2% natural habitat, 69% \pm 13% agriculture) within 1 km of the farm

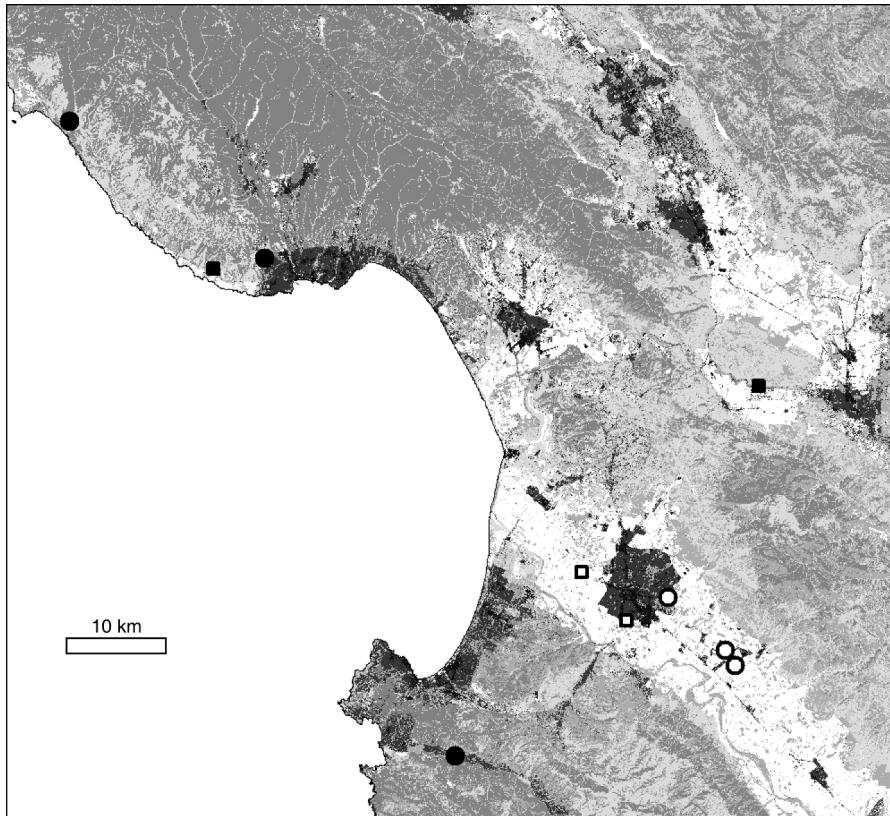


FIG. 1. Map of study sites in Salinas Valley, California, USA, and surrounding areas. White areas correspond to agricultural land, dark areas to urban/industrial/residential, and grays to different types of natural/seminatural habitat. Squares represent locally simple sites, and circles represent locally complex sites; open symbols represent agricultural matrix, while solid symbols represent natural matrix.

(established by weekly insect sampling over three years to be the most predictive scale for *B. brassicae* in this system; R. Chaplin-Kramer, P. de Valpine, N. J. Mills, and C. Kremen, *unpublished manuscript*). This gradient was fairly consistent across scales, with sites containing either >50% natural habitat or <10% natural habitat at all scales from 0.5 to 3 km. Sites with high proportions of natural or non-crop habitat in the surrounding landscape would typically be defined as “complex” (measured at scales ranging from 0.5 to 6 km; Tscharrntke et al. 2005). Complexity can refer to both the landscape and local scales, however, so we use different terminology for clarity. Sites surrounded predominantly by natural habitat are called “natural matrix” landscapes and those surrounded predominantly by agriculture are called “agricultural matrix” landscapes, referring to the dominant land use in these landscapes. Four sites in each landscape category were selected for study in 2008 (for a total sample size of $N = 8$), and one additional site in each category was included in 2009 ($N = 10$). All sites were at least 2 km apart, maintaining independence at a landscape scale defined at 1 km (R. Chaplin-Kramer, P. de Valpine, N. J. Mills, and C. Kremen, *unpublished manuscript*).

The study sites also differed in local habitat complexity. “Locally complex” farms were characterized by smaller fields (1.2–4 ha) planted with multiple (5–12) crops, and the incorporation of non-crop floral resources for beneficial insects in the form of hedgerows or weed strips along the field edge. “Locally simple” farms were large (6–12 ha) broccoli monocultures lacking any non-crop habitat within the farm itself. Two farms of each type of local category were selected for each landscape category in the 2008 study. In 2009, three locally complex farms were selected for each landscape category, but no additional locally simple farms could be added, due to limitations in farmer willingness to participate.

The landscape surrounding the farms was characterized using Geographic Information Systems (ArcGIS, version 9.3.1; Environmental Systems Research Institute, Redlands, California, USA). Aerial photographs at 1-m resolution were obtained through the National Agricultural Imagery Project (2005 true-color version; *available online*)² for the area around each farm site. The photographs were segmented using an object-based

² <http://www.apfo.usda.gov>

image analysis program with a scale parameter of 500, a shape parameter of 0.1, and a smoothness parameter of 0.5 (eCognition, version 5.0; Definiens, Alexandria, Virginia, USA). The resulting maps were classified by hand to differentiate between agricultural, and natural or seminatural habitat. Proportional areas were then computed for each land-use class at a radius of 1 km around the farm site using Hawth's tools (version 3.27; *available online*)³.

Experimental design

Broccoli plants were transplanted into pots from one-month-old starts (acquired from Growers Transplanting, Salinas, California, USA) in the greenhouse one month prior to the start of each experiment; plants were grown in identical conditions for early and late seasons to maintain consistency across time periods. The potted plants were then set out in cages (one plant per cage) at each farm site for 12 days. Cages were either closed, with all sides covered by mesh, or open, with two of the sides left open (Appendix A). Data-loggers recorded temperature and relative humidity at 10-min intervals in each of the treatments at each site to assess microclimate differences between open and closed cages (Hygrochron I-buttons; Embedded Data Systems, Lawrenceburg, Kentucky, USA). Temperature and relative humidity were similar in both types of cages ($19.5^\circ \pm 0.7^\circ\text{C}$ vs. $18.6^\circ \pm 0.7^\circ\text{C}$, and $80.6\% \pm 1.7\%$ vs. $83.9\% \pm 1.7\%$ relative humidity; means \pm SE for closed vs. open cages, respectively).

The potted broccoli plants used in this cage experiment had identical initial aphid densities, to provide a comparison of aphid growth in different locations with and without natural enemies, independent of the natural rate of aphid establishment. The one-month-old plants were inoculated with 50 aphids each, to match the normal range of 0–100 aphids per plant for plants of that age in the field (Chaplin-Kramer 2010). Infested leaves from greenhouse *B. brassicae* colonies were placed on experimental plants, and the remaining aphids were given several days to transfer from cut leaf to living plant. The aphid populations on the experimental plants reflected the age structure of the colony population, which was consistently skewed toward the younger instars (since reproductive adults produce several nymphs per day); however, first and second instar aphids were removed because wing buds could not be detected in these early stages. Alate morphs and individuals with wing buds were removed to ensure that aphids would not leave the open cages during the course of the experiment; we judged this procedure successful since few (<5%) of the aphids found in the closed cages at the end of the experiment were alate. Once the transfer was complete, aphids on the experimental plants were counted and aphids in excess of 50 were removed.

Aphids were recounted following transport of plants to the field.

The experiments were carried out in August 2008 and twice in 2009: in June, the early season before *B. brassicae* populations peak in late July or August, and again in August, the late season when aphid densities are generally high across all regions (Chaplin-Kramer 2010). Cages were arranged in groups (three per site in 2008, two per site in 2009) at the edges of broccoli fields to standardize for differences in field size (cf. Kremen et al. 2004). Each group had one closed cage, two open cages, and one sentinel cage (identical in design to the open cage, but not inoculated with aphids). This resulted in a total of 12 cages (six open, three closed, three sentinel) for each of the eight sites in 2008 ($12 \times 8 = 96$ cages total), and eight cages (four open, two closed, two sentinel) for each of the 10 sites over two time periods in 2009 ($8 \times 10 \times 2 = 160$ cages total). More open cages were included than closed or sentinel because open cages showed the greatest variance in aphid population growth in pilot studies.

The closed cages measured the population growth of aphids in absence of predation over 12 days at each site. The open cages measured the population growth of aphids when exposed to predation and/or parasitism by natural enemies. The sentinel cages measured net colonization rate, the number of aphids arriving during the course of the experiment (less the number of those lost to predation or parasitism). As aphids only colonize a plant as alate (winged) adults that can come and go over the course of an experiment, the signal of colonization is the immobile nymphs produced by these transient adults. The reproductive rate of these colonizing aphids was assumed to match (or at least correlate with) the reproductive rate found in the closed cages at that site, and therefore was used to remove the contribution of reproduction from that of colonization. Few alate adults were found on the plants at the end of the experiment (typically <5 adults per plant); these individuals when found were not included in the total to avoid double-counting. There may also have been some third-generation reproduction occurring on the sentinel plants (if aphids born from colonizing adults reached maturation during the study period), though the extent of this would be minimal, as cabbage aphid generation length is around 10 days for the field conditions in this system (Hughes 1963). The colonization measured by the uninfested sentinel plants is likely higher than would be found on the pre-infested plants in the open cages, as cabbage aphids have been found to prefer settling on unoccupied plants in avoidance of induced plant defenses (Prado and Tjallingii 2007). However, it is a useful measure of initial colonization that newly planted crops face in the field, and provides good indication of how aphid colonization differs across sites.

Plants were harvested at the end of the 12 days. Additionally, 150–250 g of leaf matter was collected at each site along 20-m transects adjacent to the cages and

³ <http://www.spatialecology.com>

moving toward the interior of the field at the beginning and end of the experiment. This provided a comparison of insect densities (per gram of wet mass plant material) in the cages vs. in the field. Experimental plants and leaf matter samples were individually bagged and brought back to the laboratory for exhaustive counting of all insect inhabitants. Natural enemies found in the open cages included syrphid fly larvae, coccinellid beetles, and various spiders, a representative though less diverse array of enemies found in the broader fields at the sites (Chaplin-Kramer 2010). However, because the open cages were designed specifically so that enemies could easily enter and leave over the course of the experiment, we did not analyze predator densities or diversity within the cages.

Pest control services

For the purposes of this experiment, pest control services were defined as the ability of resident natural enemies to constrain aphid population growth. This was measured by the proportional reduction in aphid density in the open cages as compared to the closed cages, which were free of control from predators and parasitoids. The proportional reduction in aphid density (PRD) for each site j was calculated as

$$\text{PRD}_j = D_{Oj}/D_{Cj} \quad (1)$$

where D_{Oj} is site j 's final average density of aphids on the plants in the open cages and D_{Cj} is site j 's final average density of aphids in the closed cages. This measure of PRD allows a clearer comparison between sites than absolute density (measured as aphid pressure, P , see next paragraph) because it controls for inevitable differences in aphid settlement and population growth rate among sites, such as those that might be caused by the presence of source populations or temperature differences, which strongly affects aphid growth (Dixon 1977). PRD may underestimate total mortality because it does not account for aphid colonization in the open cages, but as previously noted, the colonization measured by sentinel cages is likely overestimated and cannot be used as a proxy. Therefore, the pest reduction measured here can be considered a conservative estimate.

Aphid pressure

The experimental design also allowed for additional comparisons between sites, including factors such as pest reproduction and colonization. Net average reproductive rate (R) over 12 days for each site j was measured as the change in aphid densities within the closed cages, or

$$R_j = D_{Cj}/50 \quad (2)$$

where D_{Cj} is site j 's final average aphid density within the closed cages and 50 is the initial aphid density within all closed cages. Colonization (C) at each site j was measured as the average aphid density on the sentinel plants (D_{Sj}) at the end of the experiment, normalized by

the average reproductive rate found at that site to avoid double-counting reproduction:

$$C_j = D_{Sj}/R_j. \quad (3)$$

As previously described, this formula captures the arrival of alate adults that inhabited the plant long enough to produce nymphs but departed before the end of the experiment. Combining the total number of aphids produced from reproduction with the total number of aphids arriving from colonization provides an idea of the overall aphid pressure (P) at each site (j):

$$P_j = (D_{Cj} - 50) + C_j. \quad (4)$$

Insect densities in the surrounding field

Point-estimates of insect densities in the surrounding field were achieved by counting aphids and natural enemies found in the leaf matter collected from crops adjacent to the cages, as a basis for comparison with the information gained from the experiment. The only natural enemy occurring at high enough densities for reliable comparisons using this point-estimate method was the syrphid fly larva. For better characterization of the overall enemy community found at the field sites, additional data were used from an insect survey conducted between 2006 and 2008 at each of the sites (Chaplin-Kramer 2010; R. Chaplin-Kramer, P. de Valpine, N. J. Mills, and C. Kremen, *unpublished manuscript*). In this broader insect survey, plants were collected on a weekly basis at each site over three summer growing seasons (ranging in mass from 50 g at the beginning of the season to 1–2 kg at the end), and insects were counted in a manner similar to that used for the cage experiment. Average abundances (per gram of plant material) of syrphid larvae, coccinellid beetles, lacewings, aphid midges, and spiders were taken from this more intensively sampled, three-year data set for each site.

Analysis

All analyses were performed using generalized linear mixed-effects models (GLMM) in the statistical program R (package nlme, version 2.9.1; *available online*).⁴ Mixed-effects models were used to account for correlation between repeated measurements at the same farm sites (Gueorguieva and Krystal 2004). Site was therefore included as a random effect for all mixed-effects models for analyses on temperature, PRD, reproductive rate, colonization, aphid pressure, and enemy abundance. AIC_c scores (corrected for small sample size) were used to select the best models, determining which factors should be included in each analysis.

To determine whether there were systematic environmental differences between the local and landscape categories, maximum, minimum, and average daily

⁴ <http://www.r-project.org>

TABLE 1. Generalized linear mixed-effects model (GLMM) for fixed effects of landscape matrix, local complexity, and season on proportional reduction in aphid densities, $-\ln(\text{PRD})$, with site as a random effect.

Variable	Coefficient	SE	<i>F</i>	<i>P</i>
Intercept (agricultural-simple-early)	-0.20	0.34	-0.57	0.57
Landscape (natural)	3.36	0.43	42.16	<0.001
Local (complex)	0.65	0.26	6.26	0.041
Season (late)	0.69	0.38	5.71	0.030
Landscape \times season	-2.61	0.54	23.32	<0.001

Notes: For this and all subsequent tables, coefficients and standard errors (SE) are presented for each variable that contributed to the best model (based on AIC_c), and the reference levels of categorical variables are presented parenthetically. *P* values are also presented for all variables and interactions for the best model [$-\ln(\text{PRD}) \sim \text{landscape} \times \text{time} + \text{local}$].

temperatures recorded by the data-loggers at each site were analyzed with landscape matrix, local complexity, season, and year as fixed effects. Temperature data were further tested as predictor variables for subsequent analyses on reproductive rate, colonization, and aphid pressure.

In order to meet the assumptions of linearity for the GLMM, PRD was negative natural log (\ln) transformed for all analyses. Differences in $-\ln(\text{PRD})$ were assessed with colonization (*C*), local complexity (simple or complex), landscape matrix (natural or agricultural), season (early or late), and year (2008 or 2009) as fixed effects. Local by landscape matrix interactions were included, as were interactions between each of these factors, season and year.

Net reproductive rate ($\ln[R]$) and colonization ($\ln[C]$) were also each \ln -transformed and analyzed with several possible fixed effects and interactions, including landscape matrix, local complexity, season, year, and temperature. The presence of weedy mustard (as a potential refuge for aphids) was an additional factor used in the analysis of colonization only.

Aphid pressure (*P*) was converted to a measure of *P*/100 g of leaf material for comparison to field aphid densities, and both measures were \ln -transformed in a simple linear model to test for a correlation between these two variables. The natural logs of aphid pressure and field aphid densities were also each analyzed in mixed-effects models including the same potential variables as the analysis for net reproductive rate and colonization (see previous paragraph).

Point-estimate syrphid abundance in the surrounding field and annual average natural enemy abundances for the three years of survey data were each \ln -transformed and analyzed in mixed-effects models against landscape matrix, local complexity, and interactions between these two factors. The \ln point-estimate syrphid abundance, \ln three-year average syrphid abundance, and \ln three-year average non-syrphid natural enemy abundance were also included in mixed models (along with year and season) against $-\ln(\text{PRD})$ at each site in order to investigate whether sites characterized by higher enemy densities (both during the experiment and over a longer period preceding it) received greater pest control services.

The replicate sites in each treatment of landscape or local complexity may not be independent, as the predominant agricultural area in this system is the Salinas Valley, and the agricultural matrix sites are therefore closer to each other than to the natural matrix sites (Fig. 1). However, such spatial autocorrelation can be measured directly, with an analysis that teases apart the variation in response variables explained by space alone from the variation explained by other variables (in this case, habitat complexity at landscape or local scales). Moran's index quantifies the degree of spatial autocorrelation in the data, using the residuals from the model for each analysis in which landscape or local factors were found to be significant (following Lichstein et al. 2002, Kremen et al. 2004). We therefore computed Moran's index for any variables that responded significantly to landscape complexity.

RESULTS

Environmental differences

Average temperatures were higher in August 2008 than in August 2009 ($19.5^\circ \pm 0.7^\circ$ vs. $17.2^\circ \pm 0.3^\circ\text{C}$), but the differences in temperature between early and late season in 2009 were minor (Appendix B). Landscape and local complexity did not affect temperature.

Pest control services

There were significant local, landscape, and seasonal effects on the proportional reduction of aphid densities, PRD, as well as a significant interaction between landscape and season (Table 1, Fig. 2). Specifically, in the late season (August), the effect of local complexity in the natural matrix landscape was not significant, but locally complex sites had six times the PRD of locally simple sites in the agricultural matrix landscape ($t = 2.48$, $P = 0.031$). Similarly, there was no significant advantage of having a natural matrix at locally complex sites, but natural matrix landscapes showed a more than sixfold pest control advantage over agricultural matrix landscapes at locally simple sites ($t = 2.57$, $P = 0.026$). In contrast, local factors were not significant in the early season (June). Landscape matrix type alone impacted early-season PRD, with sites in a natural matrix exhibiting orders of magnitude higher PRD of sites in

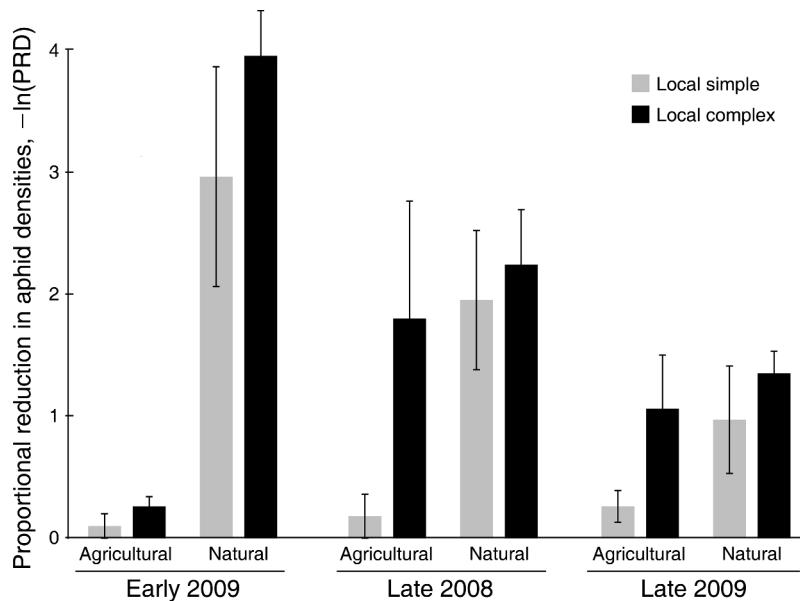


FIG. 2. The effect of landscape matrix and local complexity on the \ln -transformed proportional reduction in aphid densities, $-\ln(\text{PRD})$, for 2009 early season (June), 2008 late season (August), and 2009 late season (August). Light gray represents locally simple farms, and dark gray represents locally complex farms in agricultural vs. natural matrix landscapes. Error bars denote standard errors. Histograms are shown out of chronological order so that the two late seasons can be compared side by side.

an agricultural matrix ($t = 6.99$, $P < 0.001$). Locally complex agricultural matrix sites exhibited significant increases in PRD between early and late season ($t = 2.20$, $P = 0.049$), while PRD in natural matrix sites significantly declined over the season ($t = 4.47$, $P < 0.001$ for locally complex sites; $t = 2.49$, $P = 0.030$ for locally simple sites). As a result of these opposing trends, locally complex agricultural matrix sites reached nearly as high levels of PRD as those found in the natural matrix landscapes in the late season, but in the early season were indistinguishable from the simplest sites (locally simple agricultural matrix). Including year and colonization, $\ln(C)$, as additional variables did not improve the model.

Aphid pressure

Net reproductive rate increased with average temperatures and over the season (Fig. 3a, Table 2). Colonization was higher on farms with higher average temperatures and with mustard growing around the field (Fig. 3b, Table 2). Aphid pressure, a composite of reproductive increases and colonization, also increased with average temperature, season, and mustard, with the additional effects of year and an interaction between mustard and year (Table 2). Local and landscape complexity were not predictive of reproductive rate, colonization, or aphid pressure (Fig. 4).

There was a significant correlation between aphid pressure in experimental cages and field aphid densities (Fig. 4; $r = 0.61$, $P < 0.001$). Like aphid pressure, field aphid densities increased over the course of the season ($F = 18.04$, $df = 1, 15$, $P < 0.001$), but were only

correlated with maximum not average temperatures ($F = 8.55$, $df = 1, 15$, $P = 0.01$). No other factors were significant, including landscape or local complexity (Fig. 4).

Natural enemies

Syrphid densities in the field from point-estimates were an order of magnitude higher in natural than agricultural matrix landscapes (Fig. 5a). No syrphids were found in the early season at any farms, so the analysis was limited to data taken in the late season (Table 3). While the effect of local complexity was not significant, locally complex natural landscapes had dramatically higher syrphid abundance than locally simple or locally complex agricultural landscapes ($t = 4.71$, $P = 0.003$; $t = 4.63$, $P = 0.004$, respectively). Higher densities of syrphid larvae in the fields were associated with higher PRD (Fig. 5b; Table 4).

Mean syrphid larval abundance from the three-year survey data was several times higher in natural landscapes, but local complexity was not significant (Fig. 5c, Table 3). Pooled data for other enemies (coccinellid beetles, lacewings, aphid midges, and spiders) showed marginally significant higher abundance in natural landscapes (Fig. 5e, Table 3), but were not significant when analyzed separately. Parasitism rates were $<5\%$ (parasitized aphids per total aphids), and showed no effect of landscape or local factors. PRD increased with both syrphid densities and non-syrphid pooled enemy densities, with significant interactions with year (Fig. 5d, f, Table 4).

Spatial autocorrelation

We found no significant spatial autocorrelation between sites for PRD or any of the measures of natural enemy densities (Appendix C). That is, though all agricultural matrix sites were spatially clustered, the clustering itself was not predictive of variation in the data. The sites that were closest together were not the most similar in terms of PRD or natural enemy densities. Average temperatures were also not autocorrelated. Minimum temperatures in early season 2009 did show spatial autocorrelation ($P < 0.001$), but this effect disappeared by the late season (Appendix C).

DISCUSSION

This study suggests that habitat complexity enhances pest control services provided by natural enemies on farms, and that complexity at the local scale (crop diversity, floral resources) can substitute for that at the landscape scale (natural habitat) or vice versa, late in the season when pest populations are peaking. In the late season, sites with high local complexity and low levels of natural habitat in the landscape matrix had proportional reduction in aphid densities (PRD) equivalent to sites with low local complexity and high levels of natural habitat in the landscape matrix (Fig. 2). The PRD found at these mixed-complexity sites was only slightly lower than at the most complex sites (locally complex in natural matrix), but was substantially higher than the simplest sites (locally simple in agricultural matrix). This trend was robust across years, though more pronounced in 2008 than in 2009. The mixed-complexity sites exhibited a proportional pest reduction an average of six times greater than the simplest sites and not significantly different from the most complex sites. This refutes our initial hypothesis that complexity at both landscape and local scales would enhance pest control services relative to complexity at only one scale, but supports findings that local complexity provides greater value for natural enemy pest control in simpler (agricultural) landscapes than in complex ones (Tschamntke et al. 2005, Haenke et al. 2009, O'Rourke et al. 2011).

The apparent substitutability of complexity across scales in the late season was not evident in the early season. The lower levels of PRD found in the early season at the locally complex sites in agricultural matrix as compared to natural matrix indicate that the pest control services provided by natural enemies may be lagged at these sites. While PRD in the locally complex agricultural landscapes subsequently "caught up" to the natural landscapes by the late season, the low levels of pest reduction at these sites in the early season suggests that the ability of enemies to constrain aphid populations may be seriously compromised. Aphid densities in this system can increase by several orders of magnitude from early-season to the late-season aphid peak (Chaplin-Kramer 2010). If enemies can better constrain aphids at the low densities that occur early in the season,

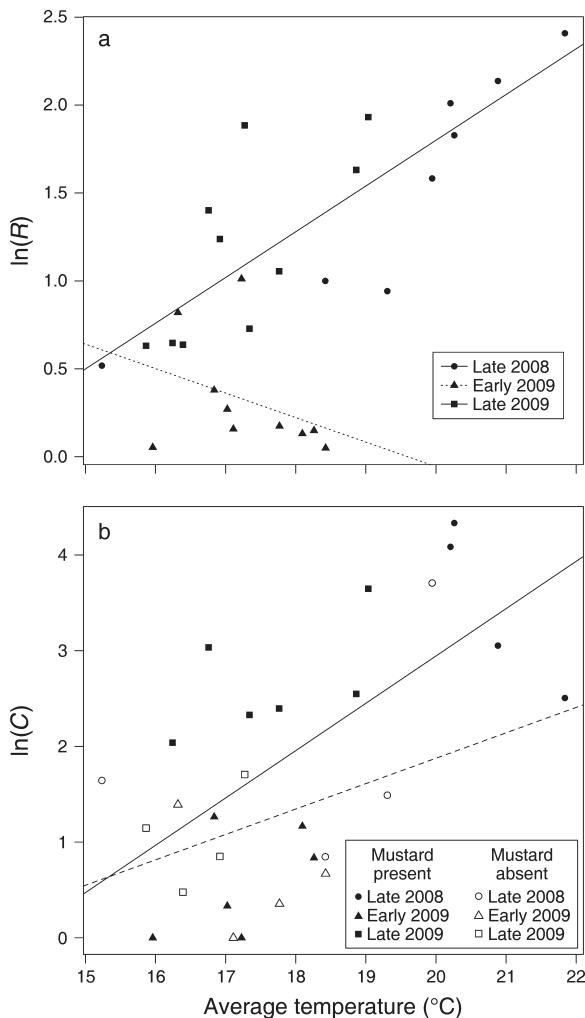


FIG. 3. Temperature effects on aphid reproduction and colonization. (a) The effect of average temperature (°C) and season on ln-transformed aphid net reproductive rate, $\ln(R)$. The solid line represents late season (both 2008 and 2009, since there was no year effect); the dashed line represents early season. (b) The effect of average temperature (°C), season, year, and the presence of mustard (probably *Brassica nigra*, but not confirmed at all sites) on ln-transformed aphid colonization, $\ln(C)$. Solid symbols and the solid line indicate that mustard was present at the site; open symbols and the dashed line indicate that mustard was absent at the site.

the subsequent aphid peak may be less pronounced. However, a delay in enemy response to aphid arrival may create a window of opportunity for rapid and unchecked aphid population growth. Indeed, previous work has demonstrated that predation during the early stages of aphid establishment determines total population and ultimately crop yields more so than predation later in the season (Östman et al. 2001).

In our study system, the locally complex agricultural matrix sites may offer enough habitat at the farm level to attract natural enemies but not enough within the surrounding landscape to sustain permanent natural

TABLE 2. GLMMs for fixed effects of mean temperatures (T), season (S), year (Y), presence of mustard (M), and/or interaction effects on net reproductive rate (R), colonization (C), and aphid pressure (P), with site as a random effect.

Variable	ln(R)			ln(C)			ln(P)		
	Coefficient	SE	P	Coefficient	SE	P	Coefficient	SE	P
Intercept	-3.10	0.78	<0.001	-79.9	52.5	0.001	0.88	2.31	0.71
Temperature	-0.12	0.11	<0.001	4.56	2.86	<0.001	0.14	0.12	<0.001
Season (late)	-5.16	1.95	<0.001	8.92	4.98	0.068			
Year (2009)				34.9	98.9	0.730	-0.94	0.41	<0.001
Mustard (present)				851	172	0.165	0.71	0.34	0.050
T × S	0.35	0.11	0.007						
T × M				-40.1	8.36	0.033			
M × Y				-878	201	0.007			
Y × T				-2.08	5.74	0.923			
T × M × Y				41.9	10.4	0.002			

Note: The intercept is for early season, 2009, mustard absent.

enemy populations. The three-year data, when examined week-to-week instead of averaged over the growing season, show that locally complex agricultural matrix sites typically do not reach syrphid densities similar to natural matrix sites until much later in the season, if at all (Appendix D; also Chaplin-Kramer 2010). In addition, though only five observations of natural enemies (primarily lacewings and spiders) were recorded in our point-estimates during the early season, all five occurred at natural matrix sites. Enemies arriving on the scene later in the season are likely drawn to the substantial prey resource in the fields, but may just be skimming off the top, rather than providing true top-

down control. The effective delivery of pest control services may rely in large part on the presence of a healthy natural enemy community on the farm early in the season, such as might occur with nearby overwintering populations, when aphid populations are small and not increasing as rapidly, and when natural enemies have the best chance of preventing populations from growing. In fact, other work in this system has shown that early-season aphid colonization allows natural enemies to establish sufficient population levels to contain aphid population levels below economic thresholds (Nieto et al. 2006). While PRD does not correspond to the absolute densities that determine

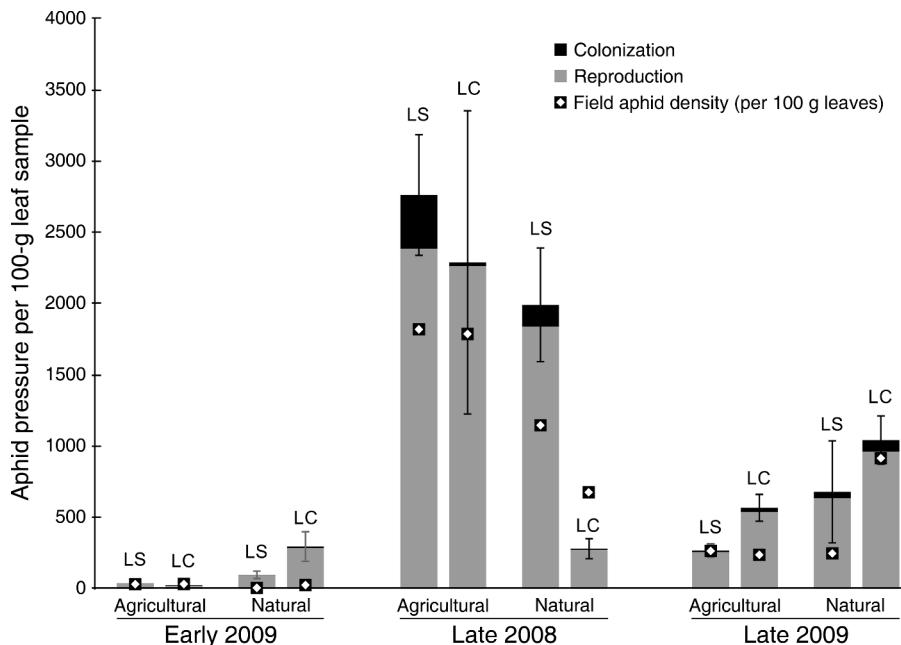


FIG. 4. Aphid pressure (P), broken down by reproduction (gray; $D_C - 50$) and colonization (black; D_S/R), as described in Eq. 4. Error bars are standard errors for $P/100\text{-g leaf sample} = (D_C - 50)/100\text{-g leaf sample} + (D_S / R) / 100\text{-g leaf sample}$. LS denotes locally simple farms, and LC denotes locally complex farms in agricultural vs. natural matrix landscapes. Diamonds represent field aphid densities per 100 g of leaves from plant material collected at each site during the experiment. Histograms are shown out of chronological order so that the two late seasons can be compared side by side.

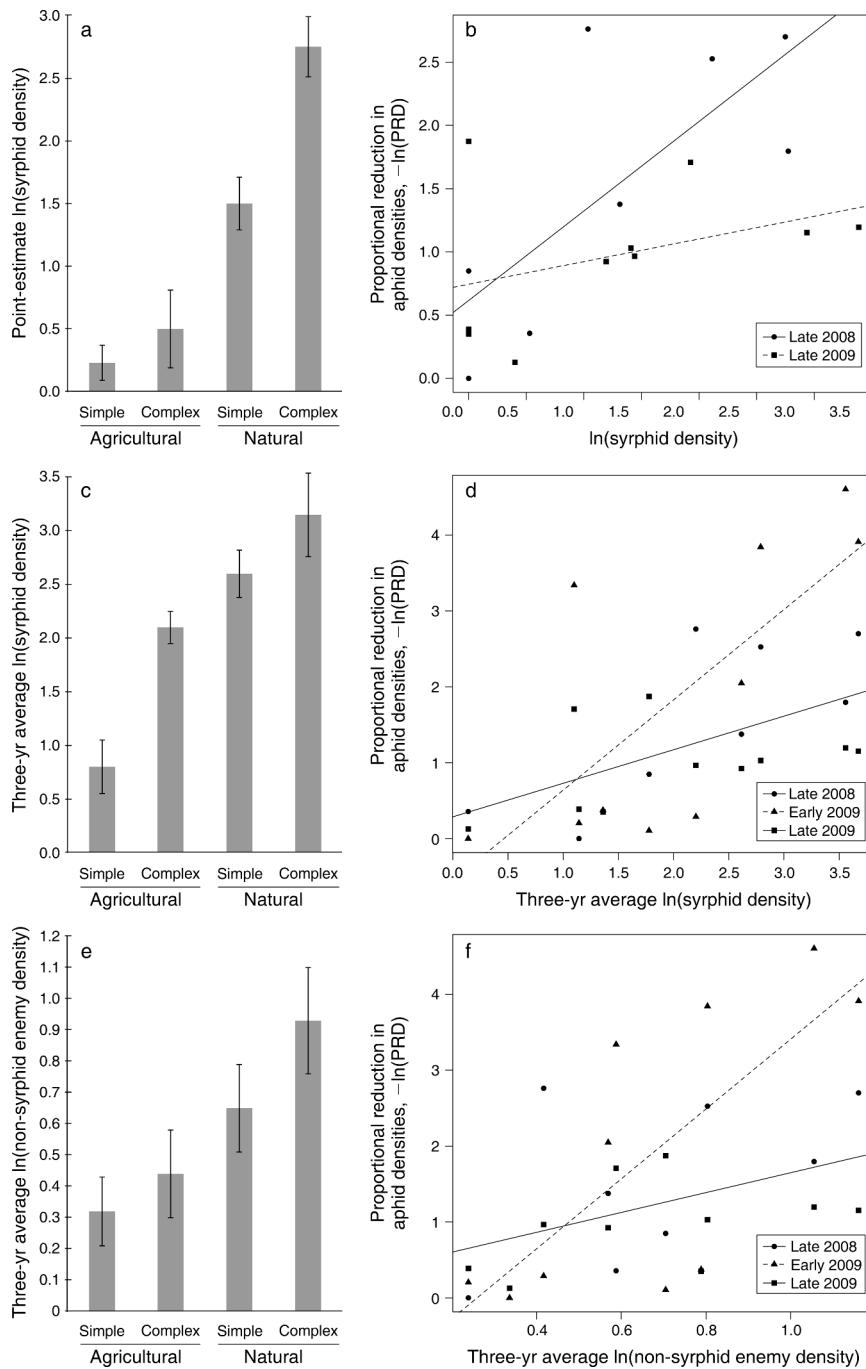


FIG. 5. (a, c, e) The effect of landscape matrix and local complexity on natural enemy densities, paired with (b, d, f) the effects of natural enemy densities on proportional reduction of aphid densities, PRD. (a) Point-estimate \ln -transformed syrphid densities per 100-g leaf sample (mean \pm SE) collected at each site during the experiment, increasing in complexity from left to right: locally simple agricultural matrix, locally complex agricultural matrix, locally simple natural matrix, locally complex natural matrix. (b) Natural log (\ln) transformed PRD vs. point-estimate \ln -transformed syrphid densities per 100-g leaf sample. Early season 2009 is not shown, as no syrphids were detected using this method. (c) Three-year average \ln -transformed syrphid densities per plant (from data collected 2006–2008) in habitats of increasing complexity left to right), with plant masses typically ranging from 100 to 1500 g over the growing season. (d) Natural log (\ln) transformed PRD vs. three-year average \ln -transformed syrphid densities per plant. (e) Three-year average \ln -transformed pooled densities of other (non-syrphid) natural enemies per plant in habitats of increasing complexity (left to right). (f) Natural log (\ln) transformed PRD vs. three-year average \ln -transformed pooled densities of other (non-syrphid) natural enemies per plant.

TABLE 3. GLMMs for fixed effects of landscape matrix (La) and/or local complexity (Lo) on ln-transformed point-estimate late-season field syrphid abundance (Sp), ln-transformed mean syrphid abundance from three-year survey data (Sm), and ln-transformed mean abundance of pooled data for all other natural enemies (E), with site as a random effect.

Variable	Sp			Sm			E		
	Coefficient	SE	<i>P</i>	Coefficient	SE	<i>P</i>	Coefficient	SE	<i>P</i>
Intercept (agricultural-simple)	0.62	0.31	0.08	1.71	0.47	0.003	0.47	0.17	0.01
La (natural)	1.83	0.38	0.002	1.32	0.50	0.02	0.38	0.17	0.06
Lo (complex)	0.66	0.39	0.13	0.70	0.50	0.21			

economic thresholds, the reduction in aphid densities provided by natural enemies associated with greater habitat complexity can be considered in terms of avoided damages. The exclusion of natural enemies has resulted in a 31% lower harvest rate for broccoli in this region (Nieto et al. 2006).

Aphid distributions in this system may be determined by many factors other than top-down control. Densities of aphids in the fields were correlated with aphid pressure in the cages, but landscape or local complexity did not significantly impact aphid densities in either field or cage (Fig. 4), despite the strong effect of complexity on PRD. However, this snapshot approach of measuring aphid densities fails to capture subtler effects of landscape complexity on population growth trajectories that may be more indicative of the level of pest control that is occurring (Chaplin-Kramer et al. 2011b), and higher temporal resolution data in this system suggest that aphid densities do decline with landscape complexity (R. Chaplin-Kramer, P. de Valpine, N. J. Mills, and C. Kremen, *unpublished manuscript*). Other factors beyond habitat complexity must be considered in order to understand and anticipate pest pressure and the contribution natural enemies can make to effective pest control in agricultural settings. Warmer temperatures enhance aphid reproductive rates (Fig. 3a), and could potentially overwhelm the control an enemy community might otherwise provide in cooler conditions. Indeed, temperature was seen to be the overriding factor determining aphid densities in the field and aphid pressure in the experimental cages in this system. Colonization, the other component of aphid pressure, was influenced by alternate host plant presence (Fig. 3b),

suggesting that the specific composition of habitat on and around the farm is important. Colonization is also presumably influenced by prevailing wind patterns, since aphids are not strong fliers (Dixon and Howard 1986). In areas of high recruitment, a constant supply of aphids replenishing those lost to predation may mask the pest control service that is simultaneously being provided by natural enemies.

Management for ecosystem services requires a knowledge of the scales at which management efforts will produce the greatest benefit (Kremen 2005). While the landscape scale appears to be more important to pest reduction in this system than the local scale, growers typically have more control over their local habitat than the surrounding landscape. On-farm management, especially the creation of habitat for natural enemies via hedgerows or insectary strips and the exclusion of alternate host plants for pests, can benefit pest control in certain circumstances. However, such management may not be sufficient to constrain pest populations below economic thresholds, and farmers may need to employ other methods to achieve the desired level of control (Andow 1990, Zehnder et al. 2007). Meanwhile, efforts to protect or restore larger tracts of habitat at the landscape scale must carefully consider which types of habitat to promote, wherever possible selecting habitat that maximizes resources for beneficial insects while minimizing its potential to serve as a refuge for pests. Restoration goals often focus on other aspects of conservation than the impact of habitat on pest control services, but conservation projects may gain more traction when all potential ecosystem services accruing

TABLE 4. GLMMs for fixed effects of natural enemy densities, season, year, and/or interactions effects on proportional reduction of aphid densities, $-\ln(\text{PRD})$, with site as a random effect.

Variable	$-\ln(\text{PRD}) \sim \text{Sp} \dots$			$-\ln(\text{PRD}) \sim \text{Sm} \dots$			$-\ln(\text{PRD}) \sim \text{E} \dots$		
	Coefficient	SE	<i>P</i>	Coefficient	SE	<i>P</i>	Coefficient	SE	<i>P</i>
Intercept (2008/late)	0.58	0.37	0.14	-0.55	0.67	0.42	-1.17	0.84	0.18
Enemy	0.74	0.22	0.05	1.19	0.29	0.004	4.56	1.15	0.01
Y (2009)	0.16	0.40	0.08						
S (late)				0.85	0.83	0.09	1.55	0.92	0.08
Enemy \times Y	-0.56	0.24	0.06						
Enemy \times S				-0.75	0.35	0.05	-3.33	1.26	0.02

Notes: Measurements for enemy densities include point-estimate late-season ln-transformed field syrphid abundance (Sp), ln-transformed mean syrphid abundance from three-year survey data (Sm), and ln(mean abundance) of pooled data for all other natural enemies (E). In the headings, ellipses indicate that the full equation includes the heading variable (Sp, Sm, or E) plus the variables listed in column 1 (see Table 2 for definitions).

from their implementation are considered (Nelson et al. 2009).

The important message for growers and land managers is that habitat complexity improves pest control services at both local and landscape scales. However, pest control services, as defined here, are not the same as pest control. Certain habitats around farm sites may promote pest population growth by influencing microclimate or providing resources to pests. Pest infestations on diverse farms and/or in natural landscapes may still occur without indicating a failure of complexity to enhance natural enemy suppression of pests; to the contrary, the pest problem could be much worse without it. In many cases, considering other variables contributing to pest distributions in addition to top-down factors will be necessary in order to achieve effective natural pest control.

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SUPPLEMENTAL MATERIAL

Appendix A

A photograph showing the design of experimental cages (*Ecological Archives* A022-104-A1).

Appendix B

Effects of year, season, landscape complexity, and local complexity on maximum, mean, and minimum temperatures (*Ecological Archives* A022-104-A2).

Appendix C

Moran's index to test for spatial autocorrelation of sites for variables that responded significantly to landscape complexity (*Ecological Archives* A022-104-A3).

Appendix D

Average weekly syrphid larvae densities from 2006 to 2008 (*Ecological Archives* A022-104-A4).