



Tools and techniques for investigating impacts of habitat complexity on biological control



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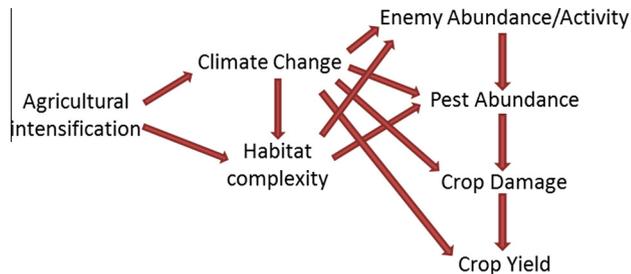
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HIGHLIGHTS

- Habitat diversity often increases the effectiveness of biological control.
- Many methods exist to measure biological control in response to habitat complexity.
- Researchers often define habitat diversity in different terms.
- New statistical, molecular, and digital technologies can aid research in this area.
- More standardized methodologies are needed for cross-study comparisons.

GRAPHICAL ABSTRACT



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ABSTRACT

Across the globe, landscapes are becoming altered as natural habitats are converted to agriculture or development. Consequently, a critical question is how changes in habitat complexity and composition might influence ecosystem services such as biological control. Although the development of new statistical, molecular, and digital technologies offers exciting opportunities to explore this issue, the appropriate usage of these tools is crucial to any successful study. This review examines the tools and techniques employed to investigate relationships between habitat complexity and biological control, and their appropriateness in different contexts. We examine various definitions of the explanatory variable, habitat complexity, and methods to experimentally measure the response variable, biological control. We conclude with a summary of the different statistical techniques available to assess linkages between habitat complexity and biological control. This review will facilitate future research on habitat complexity and biological control and will thus aid in the conservation of this valuable ecosystem service.

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1. Introduction

Current projections indicate that the global human population will exceed 9 billion people by 2050 (UN, 2009), and worldwide agricultural output will have to rapidly increase to avoid large-scale famine (Alexandratos, 1999). To meet rising demand for agricultural commodities, ever-increasing amounts of land are being cultivated. Between 1961 and 2009, global agricultural acreage

increased by 12% to 1.53 billion hectares (Fuglie and Nin-Pratt, 2012). Although conversion of land to agriculture has stagnated in the developed world, the Food and Agriculture Organization (FAO) projects that agricultural acreage will increase by another 12% in developing countries within the next 40 years (Bruinsma, 2009).

One consequence of agricultural intensification is a decline in habitat and faunal diversity (Vitousek et al., 1997; Siemann et al., 1998). This is concerning because many ecosystem services such as biological control lose functionality when biodiversity is degraded (Hooper et al., 2005; Crowder et al., 2010; Crowder and

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Jabbour, 2014). Consequently, many nations have enacted policies to promote natural habitat conservation in agroecosystems. In Europe, for example, many nations have devised “agri-environment schemes” to compensate farmers for productivity lost due to biodiversity preservation. However, habitat diversification under agri-environment schemes does not always lead to greater biodiversity (Kleijn et al., 2001, 2006). Moreover, recent meta-analyses suggest that although florally-diverse landscapes promote higher densities of invertebrate predators, pest abundances and crop yields do not always respond favorably to habitat diversification (Letourneau et al., 2011; Chaplin-Kramer et al., 2011).

These results indicate that there is much to learn about how habitat complexity influences biological control (see also Burkman and Gardiner, 2014; Dreyer and Gratton, 2014). Fortunately, recent advances in experimental and statistical techniques provide exciting new tools to investigate this topic. Molecular and digital technologies have expanded opportunities for characterizing and quantifying biological control, and recently developed statistical packages offer new tools for examining and defining habitat complexity across landscapes. Consequently, with so many new and emerging options available, choosing an approach can prove daunting. Here, we provide a comprehensive review of the experimental and analytical methodologies employed to investigate the relationships between habitat complexity and biological control. These techniques are critical to deconstruct the mechanisms by which climate change and agricultural intensification interact with biological control, both now and in the future.

2. What constitutes habitat complexity? Defining the explanatory variable

Before embarking on a study exploring effects of habitat complexity on biological control, investigators must first address the question: what does it mean for a habitat to be “complex?” There are many methods used to quantify habitat complexity, and it is important to account for these differences when making cross-study comparisons. First, complexity occurs at both local and landscape scales. Methods to augment local complexity include the preservation of field margins, intercropping, and trap-cropping, and effects of these schemes on biological control have been reviewed (Landis et al., 2000). However, comparatively few studies have examined the merits of various methods used to define habitat complexity across landscapes (often referred to as landscape complexity). Here, we outline and discuss several competing definitions of habitat complexity at a landscape scale. Moreover, we discuss the context(s) where they are appropriate, depending on the data available *a priori* to researchers (Fig. 1).

2.1. Structural habitat complexity

The majority of researchers define landscapes in terms of “structural habitat complexity”, which measures the different habitat types present in an ecosystem. One of the most common ways to characterize structural habitat complexity is to determine the percentage of semi-natural habitat in a landscape. For example, if one were interested in the diversity of natural enemies on a farm, one might calculate the proportion of natural habitat within a certain distance of the farm. The proportion of semi-natural habitat in a landscape is arguably the simplest metric of habitat complexity, and it is widely used in part because it is often correlated with Shannon's diversity index (Thies and Tschardtke, 1999; Steffan-Dewenter et al., 2002). Studies have found this metric is positively related to the abundance of natural enemy guilds including arachnids and predatory beetles (Purtauf et al., 2005; Drapela et al., 2008; Gardiner et al., 2009b, 2010). Moreover, semi-natural

habitat has been shown to be positively associated with natural enemy diversity, predation, and parasitism (Chaplin-Kramer et al., 2011).

However, the tendency to divide land cover types into discrete natural and agricultural categories, known as the habitat-matrix paradigm (Fischer and Lindenmayer, 2006), may be an oversimplification that fails to acknowledge variation among natural and agricultural habitats (Fabrigh et al., 2011). For example, Woltz et al. (2012) found low correlation between semi-natural habitat and Simpson's diversity index in landscapes in the north central United States. They hypothesized that the landscapes examined were functionally different than European landscapes, where many studies using proportion of semi-natural habitat as a measure of habitat complexity have been conducted. Fig. 2 illustrates why this metric may fail as an appropriate index of habitat complexity in some cases. Throughout Washington state, potatoes are generally grown in landscapes with 20% or less “natural” habitat. However, although the percentage of surrounding *natural* habitat is universally low, the complexity of *agricultural* habitats ranges from low to very high. In southern Washington, potatoes are often cultivated in a diverse mosaic of cropping systems, where up to 20 crops such as corn, alfalfa, apples, peas, and wheat are grown (Fig. 2a and b). In contrast, potatoes in north-central Washington are commonly grown in landscapes consisting of primarily wheat/fallow rotations (Fig. 2c and d). In this case, lumping all cultivated acreage into a “percentage agriculture” metric would ignore distinct differences between agricultural fields such as cropping type, management (organic vs. conventional), and permanence (perennial vs. annual). Consequently, a simple metric of non-agricultural habitat would not distinguish between the two landscapes illustrated in Fig. 2, despite the fact that these habitats may have dramatically different functional effects on pests and natural enemies.

Habitat complexity can be measured more directly using Simpson's or Shannon's diversity indices, patch richness (numbers of unique habitats), or patch evenness (the relative abundance distributions of habitats). Simpson's diversity index is calculated as $D = 1/\sum(p_i)^2$ where p_i is the proportion of habitat in the i th habitat category (Gardiner et al., 2009a). Shannon diversity index is computed as $-\sum p_i \times \ln(p_i)$. Both metrics increase with greater richness and evenness. Using these metrics, rather than proportion of non-crop area, provides a metric that in some cases may be more comparable across studies from different regions. For example, the potato field in Fig. 2 located in the wheat landscape has low diversity indices compared with the field in the complex landscape, despite them both having similar proportion of natural habitat. Each of these metrics can be calculated using computer packages such as Fragstats (McGarigal et al., 2012) or patch analyst (Rempel et al., 2012), which run on geographical information systems (GIS) platforms.

Finally, other authors have defined landscape categorizations more precisely, breaking down natural areas (Kruess and Tschardtke, 1994; Gardiner et al., 2009a; Werling et al., 2011) and croplands (Carriere et al., 2004, 2006) into more specific categories (e.g., forest, meadow, potatoes, wheat). This approach may be beneficial if certain crop types impact the community or process of interest, rather than broad-scale definitions of complexity. For example, Carriere et al. (2006) found that alfalfa, but not other crops, acted as sources for *Lygus hesperus* (Hemiptera: Miridae) pests into cotton fields. Had the authors simply looked at proportion of non-natural habitat, they may have missed this important source-sink effect. The biodiversity literature suggests that the presence of particular species is often more predictive of ecological processes than a broad measure of diversity (Cardinale et al., 2006), indicating that identifying effects of unique habitat types may be appropriate in many assessments of habitat complexity.

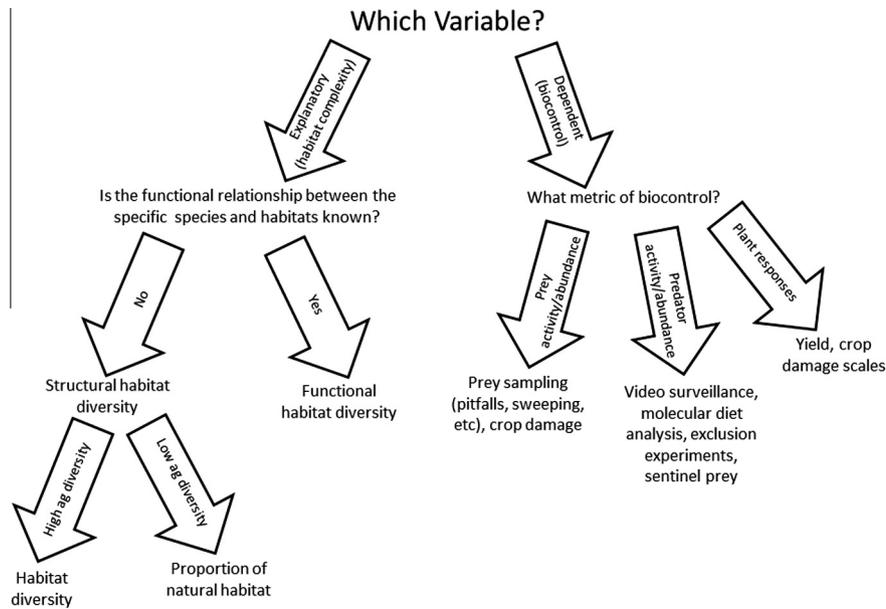


Fig. 1. Flow chart outlining the various methods used to quantify habitat complexity and biological control. Shown are the typical metrics used by researchers depending on the context of their study and data available to them.

Approaches combining multiple of these metrics may be most beneficial for analysis of the structural landscape. For example, one could use all possible definitions of complexity (proportion non-agricultural habitat, habitat diversity, habitat identity) and use model fitting to determine which definition of complexity best explains responses. In this way, each method is calculated, allowing for comparisons among studies examining effects of habitat complexity.

2.2. Functional habitat complexity

Despite the predictive power of structural habitat complexity, some authors have argued that habitat functionality may be more predictive for a particular species or group of interest (Lima and Zollner, 1996; Fahrig et al., 2011). This has led to interest in defining habitat complexity based on a measure of functional diversity. The simplest way to accomplish this is to classify habitats as sources, sinks, or neutral for a species of interest. Going further, cover types could be categorized according to their potential to provide refuge, food, oviposition habitat, or some other ecological service. How different patches of functional habitat are spatially configured may also be more predictive than simple proportions of a particular cover type, which ignore vital relationships such as connectivity (Fahrig et al., 2011). However, the inherent difficulty of delineating discrete patch boundaries can make assessments of spatial arrangement inconsistent, and factors such as inter-patch mobility can dampen effects of patch separation (Fahrig, 2013). Fahrig et al. (2011) further suggested defining functional habitat complexity separately for natural areas and agricultural areas, allowing researchers to examine which type of complexity (natural or agricultural) most affects species or processes (such as biological control) of interest.

While interest in functional habitat complexity is growing, there are several potential drawbacks. In particular, it is often difficult to know *a priori* which habitats will act as sources and sinks without a detailed understanding of the ecological interactions between habitats and species/communities. Moreover, when examining biological control services, certain habitat types may be beneficial for some natural enemy species and detrimental for others. Thus, defining habitat complexity is a complex issue that

may require different approaches depending on the ecological context and available information for the particular system of interest (Fig. 1).

2.3. Overview

Various definitions of habitat complexity provide different levels of predictive power that are context dependent. Characterizations of structural habitat diversity are most common in the ecological literature, though functional diversity may also be predictive. However, characterizing habitat functionality can be difficult if the species in question have not been extensively studied. The specific metric used in a particular study should thus be a function of the characteristics of the landscape being examined, the goal of the study, and the resources available.

3. What constitutes biological control? Defining the response variable

In Section 2 we described the various metrics used by researchers to define habitat complexity. To draw linkages with biological control, researchers must next examine biological control across study sites that reflect a gradient of complexity (Fig. 1). The activity of biological control agents at each site may then be related to the surrounding habitat. Here we discuss the various techniques used by researchers to quantify biological control across landscapes.

3.1. Measuring biological control

When describing biological control, authors typically use either one or a combination of four distinct categorizations: predator abundance/activity, herbivore abundance, crop damage, and crop yield (Letourneau et al., 2011). Although these variables are often correlated, the degree and consistency of these relationships are highly variable. Most studies have focused on natural enemy responses to landscape factors, measuring parameters such as abundance of individuals or percentage of parasitized pests in simple and complex environments (Letourneau et al., 2011). Pest abundance is another commonly used metric, but responses at the plant

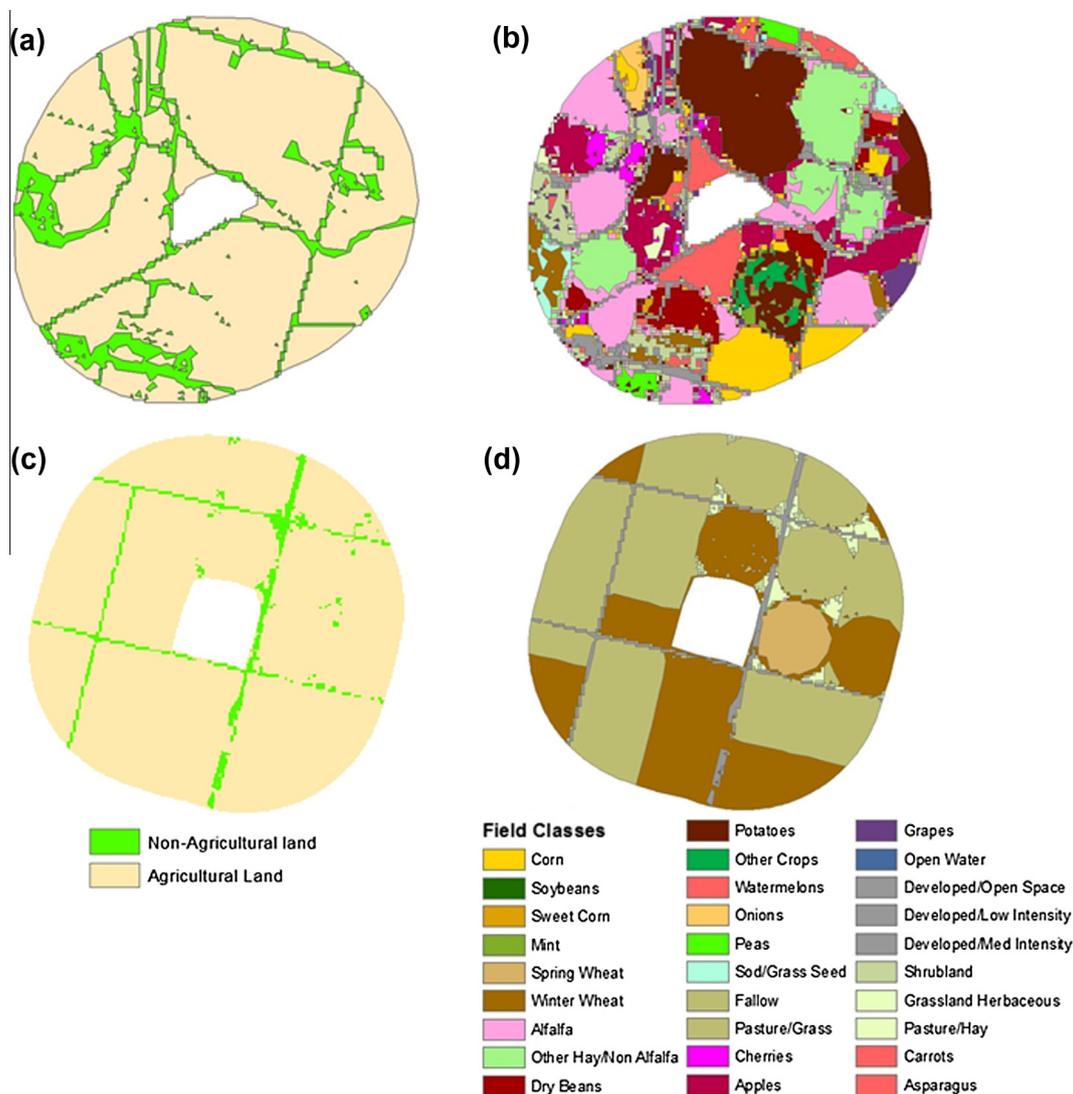


Fig. 2. Demonstration of various metrics used for habitat complexity, where different colors represent different crop types. Panels (a) and (b) depict a potato field (white) in southern Washington, with the surrounding landscape divided into (a) discrete natural and agricultural categories, and (b) more precise categories based on crop. This example illustrates how characterizing the landscape by percent agriculture may mask the habitat complexity of regions with many cropping systems. Panels (c) and (d) show a potato field (white) in north-central Washington, with the surrounding landscape divided into (c) discrete natural and agricultural categories, and (d) more precise categories based on crop type. In a relatively non-diverse agricultural environment such as this, using percent agriculture as a measure of habitat complexity is likely to be an appropriate metric.

level, such as plant damage and crop yield, are underrepresented (Cullen et al., 2008; Letourneau et al., 2011).

It should come as no surprise that arthropod-related responses are so prolific in biological control studies across landscapes since a majority of researchers performing these studies are entomologists. However, this paradigm fails to consider several key components of biological control. First, simply measuring predator and pest abundances ignores predator-mediated behavioral changes and non-consumptive effects that will not be accounted for in population tallies and may have drastic consequences for herbivory (Nelson et al., 2004; Preisser et al., 2005). Second, while it has generally been assumed that reductions in pest populations will trickle down to positively influence crop performance, herbivore abundances have demonstrated themselves to be poor indicators of crop quality and yield (Gurr et al., 2000; Letourneau et al., 2011). Ultimately, increased yields are the end goal of any well-conceived biological control program, and studies not reporting this metric sacrifice a powerful and persuasive component of their argument. However, methods such as paired exclusion-open cage studies,

deployment of sentinel prey, and molecular diet analyses still offer valuable tools to examine linkages between habitat complexity, natural enemy communities, and biological control services.

3.2. Exclusion methods

Exclusion methods, where changes in pest abundance are monitored in the absence and presence of natural enemies, are widely used to measure biological control services in the field (Kidd and Jervis, 2005). Exclusion methods that quantify biological control services also offer an important tool to assess relationships between habitat composition and management with natural enemy function (Kidd and Jervis, 2005). For example, barriers that exclude only a sub-set of natural enemies attacking a herbivore can illustrate the importance of these groups to biological control (Bogran et al., 1998; Medina and Barbosa, 2002; Gardiner and Landis, 2007; Holland et al., 2008; Xiao and Fadamiro, 2010; Rusch et al., 2013; Martin et al., 2013). When paired with population models, exclusion methods can provide valuable insight into the direct economic

impact of certain biological control agents (Östman et al., 2003; Landis et al., 2008).

Exclusion treatments can consist of cages over plant tissue or barrier traps using liquids or sticky substrates to restrict access to plants or plant parts by crawling natural enemies (Kidd and Jervis, 2005). These studies typically begin with an equal number of herbivores placed in a natural-enemy accessible treatment which is compared to survivorship and/or population growth in one or more exclusion treatments. Exclusion studies need to account for both the effectiveness of the barrier and the impact on the survivorship or population growth of the focal herbivore. For example, mesh cages can alter microclimatic factors such as light intensity, humidity, and temperature. To account for this, exclusion cages that prevent all predators are sometimes paired with sham cages with a larger mesh size or cutouts allowing predator access (e.g., Costamagna et al., 2007). For example, Medina and Barbosa (2002) used cages with varied mesh size along with sticky barriers to examine predation of large and small tussock moth (*Orgyia leucostigma*) (Lepidoptera: Lymantriidae) larvae by flying invertebrates, crawling invertebrates, and birds and flying invertebrates with treatments either excluding or accessible to all predators. Large larvae were more frequently removed in treatments allowing birds. However, results for small larvae illustrated the importance of an exclusion control, as just as many larvae disappeared from the 'no predator' exclusion than any predator-accessible treatments (Medina and Barbosa, 2002).

Gardiner et al. (2009a) used data from exclusion cages to develop a biological control services index (BSI) to compare the extent of biological control supplied to crop fields, where:

$$BSI = \frac{\sum_{n=1}^x \frac{A_{c,p} - A_{o,p}}{A_{c,p}}}{n}$$

To calculate BSI, counts of aphids on caged plants a given number of days following the initiation of the experiment (A_c) are compared with aphid counts in the presence of predators after the same amount of field exposure time (A_o), these differences are measured for each plot (p) if multiple replicate cage and open plant pairs are collected per site, summed, and divided by the number of replicates for a given site (n). Gardiner et al. (2009a) found that the soybean aphid (*Aphis glycines*) (Hemiptera: Aphididae) BSI value measured in a given site increased with landscape diversity, illustrating that diverse landscapes supplied a greater biological control service to soybean fields than simple landscapes dominated by field crops. Similarly, Woltz et al. (2012) applied the BSI index to examine how the effect of localized addition of buckwheat strips adjacent to soybean fields and the larger-scale surrounding landscape influenced *A. glycines* biological control services. Regardless of the local habitat management or surrounding landscape, aphid populations exposed to predators were significantly reduced compared to populations in exclusion cages (Woltz et al., 2012).

Despite their potential, exclusion methods have several drawbacks. First, it is often difficult to allow only desired assemblages while excluding undesired assemblages, since insect clades do not always fall into discrete size or behavioral classes. Additionally, undesired insects such as predators may need to be manually removed from cages after they are erected, which can be labor and time intensive. Furthermore, exclusion cages may also impede movement of the herbivore, which could have negative consequences depending on the particular study. For instance, if one were interested in aphid population dynamics in the presence and absence of predators, an exclusion cage may prevent not only predators but also alate aphid immigrants from colonizing the plants within. Still, exclusion methods present a suitable platform for measuring biological control as long as these limitations are considered.

3.3. Sentinel prey

Sentinel prey are often used to measure biological control activity of predators and parasitoids across landscapes (Andow, 1990; Prokrym et al., 1992; Wilson et al., 2004; Ehler, 2004, 2007; Seagraves and Yeargan, 2009). For example, Seagraves and Yeargan (2009) documented that *Coleomegilla maculata* (Coleoptera: Coccinellidae) larvae significantly reduced corn earworm (*Helicoverpa zea*) (Lepidoptera: Noctuidae) eggs on corn plants using sentinel eggs. Similarly, Ehler (2007) found that predators significantly reduced sentinel eggs of the introduced beet armyworm (*Spodoptera exigua*) (Lepidoptera: Noctuidae) in alfalfa hay fields.

Depending on the goals of a study researchers may use the actual target prey of interest or an 'artificial sentinel' organism which may or may not occur naturally in the focal habitat to serve as an indicator of biological control activity. When using sentinel prey, researchers should consider factors such as prey density, spatial arrangement, longevity of the exposure period, and whether marking or tethering is necessary for sentinels to be recognized and retrieved. Any of these factors can affect biological control measurements. For example, Steward et al. (1988) found that wasps were more likely to attack larvae punctured with an insect pin compared with un-punctured larvae. Any life stage can be used as a sentinel although eggs and pupae are the easiest and most frequently employed.

One issue with comparing sites from different geographic regions is that the predators from those sites may not share a common prey. In this situation, artificial sentinels may be particularly useful for measuring activity levels of generalist predators (Pfannenstiel and Yeargan, 2002; Werling et al., 2011; Yadav et al., 2012; Gardiner et al., 2014). Commonly used artificial sentinels include the eggs, larvae, and pupae of Diptera or Lepidoptera. For example, within an urban landscape, Gardiner et al. (2014) examined how predatory activity changes when empty, vacant lots are converted to agricultural production by placing *H. zea* eggs within each habitat. Although no difference in egg predation was detected among habitats, a significantly greater egg removal was detected in late versus early summer which may have implications for early season pest suppression within urban farms and gardens (Gardiner et al., 2014). Also using *H. zea* eggs, Werling et al. (2011) found that grassland bioenergy crops supported a threefold greater rate of biological control service over corn. Egg biological control services in bioenergy crops were also affected by surrounding landscape composition, as predation increased with the proportion of herbaceous perennial habitat present within 1.5 km of study sites (Werling et al., 2011).

Sentinel prey have been used to measure the effectiveness of habitat management on biological control services. For example, Mathews et al. (2004) showed that the addition of compost mulch in orchards significantly enhanced the abundance of alternative prey and ground-dwelling predators, but not greater predation of sentinel larvae of the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae), relative to a typically herbicide-treated orchard groundcover. Using sentinel black cutworm (*Agrotis ipsilon*) (Lepidoptera: Noctuidae) larvae, Frank and Shrewsbury (2004) documented greater biological control service within golf fairways adjacent to conservation strips versus traditional roughs. Using sentinel European corn borer (*Ostrinia nubilalis*) (Lepidoptera: Crambidae) egg masses, Bickerton and Hamilton (2012) demonstrated that intercropping with annual insectary plants enhanced the biological control service provided by *Orius insidiosus* and *C. maculata* in bell pepper. Blaauw and Isaacs (2012) combined the use of exclusion cages and sentinel prey to measure the ability of variously sized wildflower plots to attract beneficial arthropods. Within plots that ranged from 1 to 100 m² they placed sentinel soybean plants infested with *A. glycines* and found that aphid

biological control increased with plot size (Blaauw and Isaacs, 2012). Additionally, Winqvist et al. (2011) used sentinel prey to examine landscape-level effects on biological control in organic and conventional systems. Thus, the usage of sentinels is highly flexible and may be applied to a variety of situations to measure the activity of biological control agents.

Video observation can also be used to quantify the guild of natural enemies attacking sentinel prey. In some cases, the arthropods credited with providing biological control service due to their abundance in collected samples from a habitat may not be key contributors to biological control services (Frank et al., 2007; Grieshop et al., 2012). Video surveillance provides an easy and relatively inexpensive method to document natural enemy guilds, compare attack frequencies, and observe foraging behaviors (Grieshop et al., 2012).

Video surveillance methods have been applied to compare predator guilds and biological control services among habitats and microhabitats and to assess interactions such as intraguild predation. Grieshop et al. (2012) examined attack of sentinel wax worm, *Galleria mellonella* (Lepidoptera: Pyralidae), larvae placed on the soil surface of corn and grassland bioenergy crops. They found that Formicidae and Gryllidae spent more time interacting with the larvae in corn whereas ants and slugs were more common predators in the grassland systems. Similarly, Frank et al. (2007) observed tethered light brown apply moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) in vineyard canopies and at the ground surface. The diversity of predators attacking this pest varied, with seven predators feeding on larvae on the ground and only earwigs attacking larvae present in the plant canopy. However, earwigs were disproportionately more active within the canopy than on the ground resulting in similar biological control service provided to both microhabitats (Frank et al., 2007). Grieshop et al. (2012) also used video surveillance to observe predation on cranberry fruitworm, *Acrobasis vaccinii*, (Lepidoptera: Pyralidae) hibernaculæ and Japanese beetle (*Popillia japonica*) (Coleoptera: Scarabaeidae) eggs in blueberry and found that the predator complex attacking hibernaculæ varied among ground-cover treatments. Ants were the dominant predator of both prey but were observed less commonly on woodchips than bare ground, grass or landscape fabric. Gryllidae and Arachnida also exhibited varied attack frequencies for *P. japonica* eggs across ground cover treatments (Grieshop et al., 2012). Video analysis is particularly useful in systems that have not been widely studied, since no prior knowledge of predation patterns is necessary to identify putative predators.

Despite their obvious utility, the use of sentinels presents a series of issues which need to be addressed by the researcher. For instance, predation on artificially added sentinels may not always adequately reflect predation on naturally occurring insects. Sentinels can create situations in which prey type, density, or behavior does not reflect the reality of the environment. Thus, it is important for investigators to choose appropriate sentinels which will not create unrealistic situations in the field, and pairing sentinel removal experiments with the other methods described will provide a more complete picture of biological control.

3.4. Molecular diet analyses

Molecular approaches to assess predator contributions to biological control services offer an excellent opportunity to evaluate activity under natural conditions without manipulating prey or predator density or modifying field conditions (Symondson, 2002; Sheppard and Harwood, 2005). Methods such as the use of enzyme-linked immunosorbent assays (ELISA) to detect monoclonal antibodies, PCR-based methods to detect single and multiple prey taxa, and next-generation sequencing have been thoroughly

reviewed (Symondson, 2002; Sheppard and Harwood, 2005; Pompanon et al., 2012). Here we focus on what these methods have illustrated about predator contributions to biological control services in field studies and how these tools can be applied to understand the influence of habitat complexity on natural enemy activity.

Molecular methods are frequently used to determine whether particular predators feed on a target prey as well as their relative importance to prey suppression. For example, Harwood et al. (2005) used monoclonal antibodies to demonstrate that the tetragnathid spider *Pachygnatha degeeri* is a key aphid predator in wheat. The contribution of this spider to biological control services was disproportionate to its abundance, a fact that clearly is missed when trap catches or quadrat counts are assumed to translate into pest suppression. Conservation biological control relies on predator activity at low pest abundance yet it can be difficult to quantify contributions to early season suppression. Molecular methods allow for detecting early season predator feeding (Harwood et al., 2004, 2007a,b; Traugott et al., 2008; Welch and Harwood, 2014). These tools also provide an opportunity to assess unobservable interactions such as those occurring belowground (Juen and Traugott, 2007; Read et al., 2006; Thomas et al., 2009; Eitzinger et al., 2013; A'Bear et al., 2014). For example, Read et al. (2006) and Heidemann et al. (2011) examined predation of nematodes and found that Collembola and oribatid mites act as predators.

Further, molecular analysis of predator guilds can be used to quantify the importance of biological control to pest suppression. By accounting for digestion rates in predator guts, Greenstone et al. (2010) demonstrated the relative importance of several generalist and specialist arthropods feeding on Colorado potato beetle (Coleoptera: Chrysomelidae). In some cases, analysis of predator diets can also illustrate a lack of significant biological control services. Hagler et al. (2013) found little biological control service being supplied to citrus orchards to control the glassy-winged sharpshooter (Hemiptera: Cicadellidae) with only 2.28% of collected arthropods showing pest remains in their guts. However, some authors have expressed concerns about the ability of quantitative PCR to accurately portray the amount of prey consumed by a particular predator (Weber and Lundgren, 2009). This is not a trivial issue, and in many cases molecular diet analysis may solely provide presence/absence data for particular prey rather than quantitative effects on biological control.

Molecular methods can also examine the role of intraguild predation on biological control. Traugott et al. (2012) found that one-third of spiders and carabids containing aphid DNA also fed on aphid parasitoids. Examining belowground interactions, Thomas et al. (2009) found that while ground beetle larvae reduce slug abundance, higher densities of beetle larvae in field plots decreased slug control potentially due to intraguild predation. Thus, molecular methods can paint a more complete picture of predator activity than other techniques such as sentinel prey.

Molecular tools have also demonstrated effects of alternative prey on biological control (Settle et al., 1996; Agusti et al., 2003; Harwood et al., 2004; Opatovsky et al., 2012). Natural enemy behavior clearly influence prey selection and biological control; yet, without tools to analyze predator gut contents these relationships are typically measured in simple microcosms. Diet analysis can illustrate how predator behavior affects prey selection preferences and results can be linked with availability of prey across landscapes. For example, Harwood et al. (2004) compared prey selection of two linyphiid spiders that varied in web location and time spent feeding in webs. Aphid consumption by Linyphiinae increased with aphid density and was not affected by the abundance of Collembola, which serve as alternative prey (Harwood et al., 2004). However, aphid predation by Erigoninae did vary with

alternative prey abundance (Harwood et al., 2004). Opatovsky et al. (2012) found that habitat use by a predator can also affect alternative prey use. An agrobiont linyphiid spider consumed a diet dominated by non-pest Collembola, a resource common in wheat fields during the season whereas a higher proportion of the immigrant linyphiid *Enoplognatha* consumed aphids over Collembola (Opatovsky et al., 2012). Other studies have used molecular tools to demonstrate the importance of alternative prey including earthworms (Symondson et al., 2000) and thrips (Harwood et al., 2007b).

However, like all methods, molecular tools to quantify biological control services have limitations. One issue is that molecular tools may fool researchers into inferring false linkages between predators and prey, as gut content analyses do not differentiate between direct predation and other methods of ingestion such as scavenging. For instance, researchers have detected decaying, scavenged prey items in the guts of predators using both monoclonal antibodies (Calder et al., 2005) and PCR (Foltan et al., 2005; Juen and Traugott, 2005). Secondary predation may also be a source of error establishing false trophic linkages (Harwood et al., 2001; Sheppard et al., 2005). Additionally, gut content analyses require some level of *a priori* knowledge about the system before candidate predators may be identified. Other methods, such as video analysis, may be used in this regard to identify a pool of potential predator species that may subsequently be analyzed with molecular tools to determine their contributions to biological control.

As the costs of gut-content analyses continue to decline the ability to use molecular methods to compare predator contributions to biological control services across multiple sites will become more feasible. This allows for assessment of how factors such as plant community diversity, productivity, and management within a habitat influence natural niche breadth, niche partitioning, and temporal changes in diet. When coupled with other statistical and experimental techniques, molecular analysis provides a powerful tool for measuring how biological control agents respond to habitat complexity at the landscape scale.

3.5. Overview

The majority of biological control measures focus on herbivore or predator abundance and diversity, assessment of crop damage, or measures of crop yield. This section examined the value and limitations of three methods aimed to define the structure of food webs and amount of biological control activity provided to a habitat patch: paired exclusion–open cage studies, deployment of sentinel prey, and molecular diet analyses. Each of these methods allows for open-field quantification of natural enemy contributions to biological control which enables researchers to relate this ecosystem service to multiple local and landscape-scale variables. When used in conjunction with measures such as crop yield, these tools provide a powerful assessment of the influence of habitat complexity on biological control. Of these methods, molecular techniques to assess natural enemy diets are advancing most rapidly. New tools such as next generation sequencing allow researchers to recover DNA sequences from a large diversity of consumed prey within a much greater number of predators (Pompanon et al., 2012). With costs declining and methods improving these tools offer promising opportunities to further our understanding of how habitat complexity affects food web structure and function.

4. Linking biological control and habitat complexity

Once appropriate data has been collected to quantify habitat complexity and biological control (Fig. 1), an array of approaches exists to explore linkages between these variables. Analyses at

the landscape scale are often difficult to design, statistically messy, and inconclusive. Nevertheless, insights gained from such studies can prove fundamental to our understanding of landscape ecology as well as pragmatically invaluable to producers and land managers.

Perhaps the simplest approach is to pick a scale of interest and use regression models to examine how habitat complexity at this scale affects biological control in a set of focal fields. For example, Thies and Tschardt (1999) suggested that a scale of 1.5 km was most effective in predicting variation in insect communities across agricultural landscapes, and they conducted all analyses at this scale only. This framework was expanded by Thies et al. (2003), who analyzed habitat complexity using circular “rings” of varying diameters around focal fields, allowing them to determine the scale at which complexity was most predictive. For these analyses, researchers develop models at varying scales and use metrics such as R^2 , Akaike’s Information Criterion, or Bayesian Information Criterion to determine the scale that best-explains the response. An advantage of this method is that researchers can empirically determine the scale where habitat complexity has the greatest impact on biological control, without any *a priori* knowledge.

One difficulty with analyses involving habitat complexity and biological control is that data are often spatially autocorrelated. Researchers deal with autocorrelation use methods such as Moran’s I (Gardiner et al., 2009a; Werling et al., 2011), the Mantel test (Tylianakis et al., 2007), or other spatial autocorrelation procedures. Many of these procedures can be run directly through GIS. If spatial autocorrelation is not present, analyses are simplified, but when it is present there are a variety of approaches to deal with it in statistical models. Meehan et al. (2011) accounted for spatial autocorrelation in model residuals using a simultaneous autoregressive error (SAR) term and computed spatial weights using first-order neighbors. Carriere et al. (2012) dealt with this problem by computing semivariograms, and models accounted for autocorrelation in tests of significance with effective sample sizes and degrees of freedom using modified *t* and *F* tests.

One of the more effective methods for analyzing habitat complexity effects on services like biological control was developed by Carriere et al. (2004, 2006). Their technique relied on constructing a set number of concentric rings surrounding a study field and calculating areas of each habitat type in each ring. Models are then run which account for correlations in habitat types between rings and spatial correlation across regions. This allows the researcher to determine the effects of each habitat type at each scale of interest. Under both the stepwise and multiple linear regression procedures rank-based statistics are typically used because they do not require the assumption of normality. After the best model is found, the individual slope values for each predictor (crop types) indicate whether a particular crop type is a source (positive slope) or sink (negative slope) at each scale. For example, Carriere et al. (2006) found that alfalfa acted as a significant source of *Lygus* to cotton at scales up to 0.75 km, but alfalfa was not statistically significant beyond this scale. This allowed them to determine that the “zone of influence” of alfalfa as a source was 0.75 km. Thus, this method allows for a more precise calculation of how habitats influence a species or process while also determining the scale of the response.

Finally, another option for reducing the dimensionality of the landscape involves multivariate methods such as principle components analysis (PCA). These types of methods are chosen because landscapes are often delineated into individual habitat types of which there can be numerous with high correlations among them (Elliott et al., 1999; Gardiner et al., 2009a). After dimensionality of predictor variables is reduced to eigenvectors that have the highest explanatory power, they are then used in model selection techniques such as stepwise regression or AICc (Gardiner et al., 2009a). This method can be useful in analyses containing a high

number of predictors, as in cases involving many correlated habitat types. One potential drawback of PCA is that the variables created are often difficult to interpret.

Landscape-scale analyses have been greatly aided by developments in statistical software and techniques. GIS software allows for scale-dependent analyses, and model selection criteria can determine the particular scale or habitat type that is driving patterns in pests or natural enemies. Although problems such as spatial autocorrelation often complicate landscape-scale analyses, statistical solutions exist to deal with these issues. These tools offer a promising method of examining the interplay between landscape heterogeneity and biological control.

5. Conclusions and future directions

Providing food for a growing population will be one of the greatest challenges of the 21st century. Effective suppression of agricultural pests will become increasingly important as farmers strive to boost agricultural productivity in a changing world while limiting inputs and costs. Understanding how landscape-level environmental factors influence agricultural pests and crop damage is thus crucial to ensuring global food security. This is a burgeoning area of research, and despite recent advances there is still much to learn.

Individual farms and landscapes each present a different challenge, and determining how to measure biological control and habitat complexity is context dependent. Habitat functionality, agricultural diversity, and geographic scope are just a few of the factors to consider when designing an effective landscape-level study. Additionally, choosing an appropriate response variable depends on the target audience and goal of the particular study. Research in agricultural landscape ecology could be more effective, however, if researchers used similar metrics across studies. Despite these challenges, recent advances in computing power and statistical packages have provided valuable new tools to researchers in this field. This review provides an overview of these methods in an effort to make studies across regions more comparable.

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References

- A'Bear, A.D., Johnson, S., Jones, H., 2014. Putting the 'upstairs-downstairs' into ecosystem service. what can aboveground–belowground ecology tell us? *Biol. Control* 75, 97–107.
- Agusti, N., Shayler, S.P., Harwood, J.D., Vaughan, I.P., Sunderland, K.D., Symondson, W.O.C., 2003. Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. *Mol. Ecol.* 12, 3467–3475.
- Alexandratos, N., 1999. World food and agriculture: outlook for the medium and longer term. *Proc. Natl. Acad. Sci. U.S.A.* 96, 5908–5914.
- Andow, D.A., 1990. Characterization of predation on egg masses of *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Ann. Entomol. Soc. Am.* 83, 482–486.
- Bickerton, M.W., Hamilton, G.C., 2012. Effects of intercropping with flowering plants on predation of *Ostrinia nubilalis* (Lepidoptera: Crambidae) eggs by generalist predators in bell peppers. *Environ. Entomol.* 41, 612–620.
- Blaauw, B.R., Isaacs, R., 2012. Larger wildflower plantings increase natural enemy density, diversity, and biological control of sentinel prey, without increasing herbivore density. *Ecol. Entomol.* 37, 386–394.
- Bogran, C.E., Obrycki, J.J., Cave, R., 1998. Assessment of biological control of *Bemisia tabaci* (Homoptera: Aleyrodidae) on common bean in Honduras. *Fla. Entomol.* 81, 384–395.
- Bruinsma, J., 2009. The resource outlook to 2050: By how much do land, water and crop yields need to increase by 2050? In: Expert Meeting on How to Feed the World in 2050, United Nations FAO, Rome, Italy. <http://ftp.fao.org/docrep/fao/012/ak971e/ak971e00.pdf>.
- Burkman, C.E., Gardiner, M.M., 2014. Urban greenspace design and landscape context influence natural enemy community composition and function. *Biol. Control* 75, 58–67.
- Calder, C.R., Harwood, J.D., Symondson, W.O.C., 2005. Detection of scavenged material in the guts of predators using monoclonal antibodies: a significant source of error in measurement of predation? *Bull. Entomol. Res.* 95, 57–62.
- Cardinale, B.J., Srivastava, D.S., Duffy, J.E., Wright, J.P., Downing, A.L., Sankaran, M., Jousseau, C., 2006. Effects of biodiversity on the functioning of trophic groups and ecosystems. *Nature* 443, 989–992.
- Carriere, Y., Dutilleul, P., Ellers-Kirk, C., Pedersen, B., Haller, S., Antilla, L., Dennehy, T.J., Tabashnik, B.E., 2004. Sources, sinks, and the zone of influence of refuges for managing insect resistance to Bt crops. *Ecol. Appl.* 14, 1615–1623.
- Carriere, Y., Ellsworth, P.C., Dutilleul, P., Ellers-Kirk, C., Barkley, V., Antilla, L., 2006. A GIS-based approach for areawide pest management: the scales of *Lygus hesperus* movements to cotton from alfalfa, weeds, and cotton. *Entomol. Exp. Appl.* 118, 203–210.
- Carriere, Y., Ellers-Kirk, C., Hartfield, K., Larocque, G., Degain, B., Dutilleul, P., Dennehy, T.J., Marsh, S.E., Crowder, D.W., Li, X.C., Ellsworth, P.C., Naranjo, S.E., Palumbo, J.C., Fournier, A., Antilla, L., Tabashnik, B.E., 2012. Large-scale, spatially-explicit test of the refuge strategy for delaying insecticide resistance. *Proc. Natl. Acad. Sci. U.S.A.* 109, 775–780.
- Chaplin-Kramer, R., O'Rourke, M.E., Blitzer, E.J., Kremen, C., 2011. A meta-analysis of crop pest and natural enemy response to landscape complexity. *Ecol. Lett.* 14, 922–932.
- Costamagna, A.C., Landis, D.A., Difonzo, C.D., 2007. Suppression of soybean aphid by generalist predators results in a trophic cascade in soybeans. *Ecol. Appl.* 17, 441–451.
- Crowder, D.W., Jabbour, R., 2014. Relationships between biodiversity and biological control in agroecosystems: current status and future challenges. *Biol. Control* 75, 8–17.
- Crowder, D.W., Northfield, T.D., Strand, M.R., Snyder, W.E., 2010. Organic agriculture promotes evenness and natural pest control. *Nature* 466, 109–112.
- Cullen, R., Warner, K.D., Jonsson, M., Wratten, S.D., 2008. Economics and adoption of conservation biological control. *Biol. Control* 45, 272–280.
- Drapela, T., Moser, D., Zaller, J.G., Frank, T., 2008. Spider assemblages in winter oilseed rape affected by landscape and site factors. *Ecography* 31, 254–262.
- Dreyer, J., Gratton, C., 2014. Habitat linkages in conservation biological control: lessons from the land–water interface. *Biol. Control* 75, 68–76.
- Ehler, L.E., 2004. An evaluation of some natural enemies of *Spodoptera exigua* on sugarbeet in northern California. *Biol. Control* 49, 121–135.
- Ehler, L.E., 2007. Impact of native predators and parasites on *Spodoptera exigua*, an introduced pest of alfalfa hay in northern California. *Biol. Control* 52, 323–338.
- Eitzinger, B., Micit, A., Korner, M., Traugott, M., Scheu, S., 2013. Unveiling soil food web links: new PCR assays for detection of prey DNA in the gut of soil arthropod predators. *Soil Biol. Biochem.* 57, 943–945.
- Elliott, N.C., Kieckhefer, R.W., Lee, J.H., French, B.W., 1999. Influence of within-field and landscape factors on aphid predator populations in wheat. *Landscape Ecol.* 14, 239–252.
- Fahrig, L., 2013. Rethinking patch size and isolation effects: the habitat amount hypothesis. *J. Biogeogr.* 40, 1649–1663.
- Fahrig, L., Baudry, J., Brotons, L., Burel, F.G., Crist, T.O., Fuller, R.J., Sirami, C., Siriwardena, G.M., Martin, J.L., 2011. Functional landscape heterogeneity and animal biodiversity in agricultural landscapes. *Ecol. Lett.* 14, 101–112.
- Fischer, J., Lindenmayer, D.B., 2006. Beyond fragmentation: the continuum model for fauna research and conservation in human-modified landscapes. *Oikos* 112, 473–480.
- Foltan, P., Sheppard, S., Konvicka, M., Symondson, W.O.C., 2005. The significance of facultative scavenging in generalist predator nutrition: detecting decayed prey in the guts of predators using PCR. *Mol. Ecol.* 14, 4147–4158.
- Frank, S.D., Shrewsbury, P.M., 2004. Effect of conservation strips on the abundance and distribution of natural enemies and predation of *Agrotis ipsilon* (Lepidoptera: Noctuidae) on golf course fairways. *Environ. Entomol.* 33, 1662–1672.
- Frank, S.D., Wratten, S.D., Sandhu, H.S., Shrewsbury, P.M., 2007. Video analysis to determine how habitat strata affects predator diversity and predation of *Epiphyas postvittana* (Lepidoptera: Tortricidae) in a vineyard. *Biol. Control* 41, 230–236.
- Fuglie, K., Nin-Pratt, A., 2012. A changing global harvest. In: 2012 Global Food Policy Report. International Food Policy Research Institute, pp. 15–27.
- Gardiner, M.M., Landis, D.A., 2007. Impact of intraguild predation by adult *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Hemiptera: Aphididae) biological control in cage studies. *Biol. Control* 40, 386–395.
- Gardiner, M.M., Landis, D.A., Gratton, C., DiFonzo, C.D., O'Neal, M., Chacon, J.M., Wayo, M.T., Schmidt, N.P., Mueller, E.E., Heimpel, G.E., 2009a. Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. *Ecol. Appl.* 19, 143–154.
- Gardiner, M.M., Landis, D.A., Gratton, C., Schmidt, N., O'Neal, M., Mueller, E., Chacon, J., Heimpel, G.E., DiFonzo, C.D., 2009b. Landscape composition influences patterns of native and exotic lady beetle abundance. *Divers. Distrib.* 15, 554–564.
- Gardiner, M.M., Landis, D.A., Gratton, C., Schmidt, N., O'Neal, M., Mueller, E., Chacon, J., Heimpel, G.E., 2010. Landscape composition influences the activity density of Carabidae and Arachnida in soybean fields. *Biol. Control* 55, 11–19.
- Gardiner, M.M., Prajzner, S.P., Burkman, C.E., Albro, S., Grewal, P.S., 2014. Vacant land conversion to community gardens: influences on generalist arthropod predators and biological control services in urban greenspaces. *Urban Ecosyst.* <http://dx.doi.org/10.1007/s11252-013-0303-6>.
- Greenstone, M.H., Szendrei, Z., Payton, M.E., Rowley, D.L., Coudron, T.C., Weber, D.C., 2010. Choosing natural enemies for conservation biological control: use of the

- prey detectability half-life to rank key predators of Colorado potato beetle. *Entomol. Exp. Appl.* 136, 97–107.
- Griehner, M., Werling, B., Buehrer, K., Perrone, J., Isaacs, R., Landis, D., 2012. Big brother is watching: studying insect predation in the age of digital surveillance. *Am. Entomol.* 58 (3), 172–182.
- Gurr, G.M., Wratten, S.D., Barbosa, P., 2000. Success in conservation biological control of arthropods. In: Gurr, G., Wratten, S. (Eds.), *Biological Control: Measures of Success*. Springer, Netherlands, pp. 105–132.
- Hagler, J.R., Blackmer, F., Symondson, W.O.C., Groves, R.L., Morse, J.G., Johnson, M.W., 2013. Gut content examination of the citrus predator assemblage for the presence of *Homalodisca vitripennis* remains. *Biol. Control* 58, 341–349.
- Harwood, J.D., Phillips, S.W., Sunderland, K.D., Symondson, W.O.C., 2001. Secondary predation: quantification of food chain errors in an aphid–spider–carabid system using monoclonal antibodies. *Mol. Ecol.* 10, 2049–2057.
- Harwood, J.D., Sunderland, K.D., Symondson, W.O.C., 2004. Prey selection by linyphiid spiders: molecular tracking of the effects of alternative prey on rates of aphid consumption in the field. *Mol. Ecol.* 13, 3549–3560.
- Harwood, J.D., Sunderland, K.D., Symondson, W.O.C., 2005. Monoclonal antibodies reveal the potential of the tetragnathid spider *Pachygnatha degeeri* (Araneae: Tetragnathidae) as an aphid predator. *Bull. Entomol. Res.* 95, 161–167.
- Harwood, J.D., Bostrom, M.R., Hladilek, E.E., Wise, D.H., Obrycki, J.J., 2007a. An order-specific monoclonal antibody to Diptera reveals the impact of alternative prey on spider feeding behavior in a complex food web. *Biol. Control* 41, 397–407.
- Harwood, J.D., Desneux, N., Yoo, H.J.S., Rowley, D.L., Greenstone, M.H., Obrycki, J.J., O’Neil, R.J., 2007b. Tracking the role of alternative prey in soybean aphid predation by *Orius insidiosus*: a molecular approach. *Mol. Ecol.* 16, 4390–4400.
- Heidemann, K., Scheu, S., Ruess, L., Maraun, M., 2011. Molecular detection of nematode predation and scavenging in oribatid mites: laboratory and field experiments. *Soil Biol. Biochem.* 43, 2229–2236.
- Holland, J.M., Oaten, H., Southway, S., Moreby, S., 2008. The effectiveness of field margin enhancement for cereal aphid control by different natural enemy guilds. *Biol. Control* 47, 71–76.
- Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., Lawton, J.H., Lodge, D.M., Loreau, M., Naeem, S., Schmid, B., Setälä, H., Symstad, A.J., Vandermeer, J., Wardle, D.A., 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monogr.* 75, 3–35.
- Juen, A., Traugott, M., 2005. Detecting predation and scavenging by DNA gut-content analysis: a case study using a soil insect predator–prey system. *Oecologia* 142, 344–352.
- Juen, A., Traugott, M., 2007. Revealing species-specific trophic links in soil food webs: molecular identification of scarab predators. *Mol. Ecol.* 16, 1545–1557.
- Kidd, N.A.C., Jervis, M.A., 2005. Population dynamics. In: Jervis, M.A. (Ed.), *Insect Natural Enemies, A Practical Perspective*. Springer, Netherlands, pp. 435–523.
- Kleijn, D., Berendse, F., Smit, R., Gilissen, N., 2001. Agri-environment schemes do not effectively protect biodiversity in Dutch agricultural landscapes. *Nature* 413, 723–725.
- Kleijn, D., Baquero, R.A., Clough, Y., Diaz, M., De Esteban, J., Fernandez, F., Gabriel, D., Herzog, F., Holzschuh, A., Jöhl, R., Knop, E., Kruess, A., Marshall, E.J.P., Steffan-Dewenter, I., Tschamtkke, T., Verhulst, J., West, T.M., Yela, J.L., 2006. Mixed biodiversity benefits of agri-environment schemes in five European countries. *Ecol. Lett.* 9, 243–254.
- Kruess, A., Tschamtkke, T., 1994. Habitat fragmentation, species loss, and biological control. *Science* 264, 1581–1584.
- Landis, D.A., Wratten, S.D., Gurr, G.M., 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annu. Rev. Entomol.* 45, 175–201.
- Landis, D.A., Gardiner, M.M., van der Werf, W., Swinton, S.M., 2008. Increasing corn for biofuel production reduces biocontrol services in agricultural landscapes. *PNAS* 105, 20552–20557.
- Letourneau, D.K., Armbrrecht, I., Rivera, B.S., Lerma, J.M., Carmona, E.J., Daza, M.C., Escobar, S., Galindo, V., Gutierrez, C., Lopez, S.D., Mejia, J.L., Rangel, A.M.A., Rangel, J.H., Rivera, L., Saavedra, C.A., Torres, A.M., Trujillo, A.R., 2011. Does plant diversity benefit agroecosystems? A synthetic review. *Ecol. Appl.* 21, 9–21.
- Lima, S.L., Zollner, P.A., 1996. Towards a behavioral ecology of ecological landscapes. *Trends Ecol. Evol.* 11, 131–135.
- Martin, E.A., Reineking, B., Seo, B., Steffan-Dewenter, I., 2013. Natural enemy interactions constrain pest control in complex agricultural landscapes. *PNAS* 110, 5534–5539.
- Mathews, C.R., Bottrell, D.G., Brown, M.W., 2004. Habitat manipulation of the apple orchard floor to increase ground-dwelling predators and predation of *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). *Biol. Control* 30, 265–273.
- McGarigal, K., Cushman, S.A., Ene, E., 2012. FRAGSTATS v4: Spatial Pattern Analysis Program for Categorical and Continuous Maps. Computer Software Program Produced by the authors at the University of Massachusetts, Amherst. Available at the following web site: <http://www.umass.edu/landeco/research/fragstats/fragstats.html>.
- Medina, R.F., Barbosa, P., 2002. Predation of small and large *Orgyia leucostigma* (J.E. Smith) (Lepidoptera: Lymantriidae) larvae by vertebrate and invertebrate predators. *Environ. Entomol.* 31, 1097–1102.
- Meehan, T.D., Werling, B.P., Landis, D.A., Gratten, C., 2011. Agricultural landscape simplification and insecticide use in the Midwestern United States. *Proc. Natl. Acad. Sci. U.S.A.* 108, 11500–11505.
- Nelson, E.H., Matthews, C.E., Rosenheim, J.A., 2004. Predators reduce prey population growth by inducing changes in prey behavior. *Ecology* 85, 1853–1858.
- Opatovsky, I., Chapman, E.G., Weintraub, P.G., Lubin, Y., Harwood, J.D., 2012. Molecular characterization of the differential role of immigrant and agrobiont generalist predators in pest suppression. *Biol. Control* 63, 25–30.
- Östman, Ö., Ekbom, B., Bengtsson, J., 2003. Yield increase attributable to aphid predation by ground-living polyphagous natural enemies in spring barley in Sweden. *Ecol. Econ.* 45, 149–158.
- Pfannenstiel, R.S., Yeargan, K.V., 2002. Identification and diel activity patterns of predators attacking *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs in soybean and sweet corn. *Environ. Entomol.* 31, 232–241.
- Pompanon, F., Deagle, B.E., Symondson, W.O.C., Brown, D.S., Jarman, S.N., Taberlet, P., 2012. Who is eating what: diet assessment using next generation sequencing. *Mol. Ecol.* 21, 1931–1950.
- Preisser, E.L., Bolnick, D.L., Benard, M.F., 2005. Scared to death? The effects of intimidation and consumption in predator–prey interactions. *Ecology* 86, 501–509.
- Prokrym, D.R., Andow, D.A., Ciborowski, J.A., Sreenivasam, D.D., 1992. Suppression of *Ostrinia-Nubilalis* by *Trichogramma-Nubilale* in sweet corn. *Entomol. Exp. Appl.* 64, 73–85.
- Purtauf, T., Roschewitz, I., Dauber, J., Thies, C., Tschamtkke, T., Wolters, V., 2005. Landscape context of organic and conventional farms: influences on carabid beetle diversity. *Agric. Ecosyst. Environ.* 108, 165–174.
- Read, D.S., Sheppard, S.K., Bruford, M.W., Glen, D.M., Symondson, W.O.C., 2006. Molecular detection of predation by soil micro-arthropods on nematodes. *Mol. Ecol.* 15, 1963–1972.
- Rempel, R.S., Kaukinen D., Carr A.P., 2012. Patch Analyst and Patch Grid. Ontario Ministry of Natural Resources. Centre for Northern Forest Ecosystem Research, Thunder Bay, Ontario.
- Rusch, A., Bommarco, R., Jonsson, M., Smith, H.G., Ekbom, B., 2013. Flow and stability of natural pest control services depend on complexity and crop rotation at the landscape scale. *J. Appl. Ecol.* 50, 345–354.
- Seagraves, M.P., Yeargan, K.V., 2009. Importance of predation by *Coleomegilla maculata* larvae in the natural control of the corn earworm in sweet corn. *Biol. Control Sci. Technol.* 19, 1067–1079.
- Settle, W.H., Ariawan, H., Astuti, E.T., Cahyana, W., Hakim, A.L., Hindayana, D., Lestari, A.S., Pajarningsih, 1996. Managing tropical rice pests through conservation of generalist natural enemies. *Ecology* 77, 75–88.
- Sheppard, S.K., Harwood, J.D., 2005. Advances in molecular ecology: tracking trophic links through predator–prey food-webs. *Funct. Ecol.* 19, 751–762.
- Sheppard, S.K., Bell, J., Sunderland, K.D., Fenlon, J., Skervin, D., Symondson, W.O.C., 2005. Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. *Mol. Ecol.* 14, 4461–4468.
- Siemann, E., Tilman, D., Haarstad, J., Ritchie, M., 1998. Experimental tests of the dependence of arthropod diversity on plant diversity. *Am. Nat.* 152, 738–750.
- Steffan-Dewenter, I., Munzenberg, U., Burger, C., Thies, C., Tschamtkke, T., 2002. Scale-dependent effects of landscape context on three pollinator guilds. *Ecology* 83, 1421–1432.
- Steward, V.B., Smith, K.G., Stephen, F.M., 1988. Predation by wasps on lepidopteran larvae in an oak forest canopy. *Ecol. Entomol.* 13, 81–86.
- Symondson, W.O.C., 2002. Molecular identification of prey in predator diets. *Mol. Ecol.* 11, 627–641.
- Symondson, W.O.C., Glen, D.M., Erickson, M.L., Liddell, J.E., Langdon, C.J., 2000. Do earthworms help to sustain the slug predator *Pterostichus melanarius* (Coleoptera: Carabidae) within crops? Investigations using monoclonal antibodies. *Mol. Ecol.* 9, 1279–1292.
- Thies, C., Tschamtkke, T., 1999. Landscape structure and biological control in agroecosystems. *Science* 285, 893–895.
- Thies, C., Steffan-Dewenter, I., Tschamtkke, T., 2003. Effects of landscape context on herbivory and parasitism at different spatial scales. *Oikos* 101, 18–25.
- Thomas, R.S., Harwood, J.D., Glen, D.M., Symondson, W.O.C., 2009. Tracking predator density dependence and subterranean predation by carabid larvae on slugs using monoclonal antibodies. *Ecol. Entomol.* 34, 569–579.
- Traugott, M., Bell, J.R., Broad, G.R., Powell, W., Van Veen, J.F., Vollhardt, I.M.G., Symondson, W.O.C., 2008. Endoparasitism in cereal aphids: molecular analysis of a whole parasitoid community. *Mol. Ecol.* 17, 3928–3938.
- Traugott, M., Bell, J.R., Raso, L., Sint, D., Symondson, W.O.C., 2012. Generalist predators disrupt parasitoid aphid control by direct and coincidental intraguild predation. *Bull. Entomol. Res.* 102, 239–247.
- Tylianakis, J.M., Tschamtkke, T., Lewis, O.T., 2007. Habitat modification alters the structure of tropical host–parasitoid food webs. *Nature* 445, 202–205.
- United Nations Department of Economic and Social Affairs, 2009. World population trends. In: *World Population Prospects: The 2008 Revision*. United Nations Publications, New York, p. 3.
- Vitousek, P.M., Mooney, H.A., Lubchenco, J., Melillo, J.M., 1997. Human domination of earth’s ecosystems. *Science* 277, 494–499.
- Weber, D.C., Lundgren, J.G., 2009. Detection of predation using qPCR: effect of prey quantity, elapsed time, chaser diet, and sample preservation on detectable quantity of prey DNA. *J. Insect Sci.* 9, 1–12.
- Welch, K.D., Harwood, J.D., 2014. Temporal dynamics of natural enemy–pest interactions in a changing environment. *Biol. Control* 75, 18–27.
- Werling, B.P., Meehan, T.D., Robertson, B.A., Gratten, C., Landis, D.A., 2011. Biological control potential varies with changes in biofuel-crop plant communities and landscape perennality. *Global Change Biol. Bioenergy* 3, 347–359.
- Wilson, A.P., Hough-Goldstein, J.A., Vangessel, M.J., Pesek, J.D., 2004. Effects of varying weed communities in corn on European corn borer, *Ostrinia nubilalis* (Hubner) (Lepidoptera: Crambidae), oviposition, and egg mass predation. *Environ. Entomol.* 33, 320–327.

- Winqvist, C., Bengtsson, J., Aavik, T., Berendse, F., Clement, L.W., Eggers, S., et al., 2011. Mixed effects of organic farming and landscape complexity on farmland biodiversity and biological control potential across Europe. *J. Appl. Ecol.* 48, 570–579.
- Woltz, J.M., Isaacs, R., Landis, D.A., 2012. Landscape structure and habitat management differentially influence insect natural enemies in an agricultural landscape. *Agric. Ecosyst. Environ.* 152, 40–49.
- Xiao, Y.F., Fadamiro, H.Y., 2010. Exclusion experiments reveal relative contributions of natural enemies to mortality of citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Alabama satsuma orchards. *Biol. Control* 54, 189–196.
- Yadav, P., Duckworth, K., Grewal, P.S., 2012. Habitat structure influences below ground biological control services: a comparison between urban gardens and vacant lots. *Landscape Urban Plann.* 104, 238–244.