UNTANGLING THE ROLE OF GENERALIST PREDATORS IN BIOLOGICAL CONTROL IN ORGANIC VEGETABLE AGRICULTURE

by

JOSEPH M TAYLOR

(Under the Direction of William E. Snyder)

ABSTRACT

Generalist predators can be a useful component of biological control, but their role is complex and often context dependent. Their broader feeding allows for higher persistence in the dire conditions of an agricultural setting where prey abundance and diversity may be relatively low. The purpose of this research is to both evaluate new methods of investigating realized diets as well as evaluating how local and landscape factors influence the diet items of generalist predators in the field. The carabid beetle *Pterostichus melanarius* was used as an example of one such generalist. We evaluated shotgun metagenomics in comparison to typical metabarcoding methods to determine what prey items, pests or non-target, that *P. melanarius* were consuming. A general bioinformatic pipeline involving contig formation using established metagenome assemblers was also created. Shotgun metagenomics identified a broader suite of diet items, but the increased DNA requirements limited the power of statistical analysis. In addition, local landscape and management features were tested for how they impacted both the number and identity of diet items. On the same organic farms, insect populations were observed, and the influence of both local and landscape level factors were tested for predictive power. Overall community metrics and natural enemy taxa were influenced by local ground cover while brassica pests only meaningfully responded to crop size. Finally, the top-down pressure of birds on the arthropod pest and predator community was evaluated using a combination of fecal diet analysis and small-scale exclusions. We also examined whether landscape level features influenced bird predation pressure. We found that brassica pests and arthropod predators were relatively uncommon prey for birds. Accordingly, bird access yielded higher, rather than lower aphid abundances but little impact on other arthropod abundances. The broad conclusion drawn from this body of research is that generalist predators (both arthropod and avian) both benefit pest suppression by eating herbivores and disrupt it by eating other predators, and in sum these positive and negative effects often counterbalance one another to yield no clear net change in pest abundance. Nonetheless, broad landscape studies, molecular techniques, and on farm experimentation can complement one another to form a complete picture that generates policy recommendations.

INDEX WORDS: *Pterostichus melanarius*, molecular diet analysis, trophic cascade, ecology, landscape ecology, agricultural entomology, biological control.

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DEDICATION

This dissertation is dedicated to my family. Brandon, thanks for always lending an ear in the wee hours of the morning to discuss ideas and letting me talk things through. Mom, dad, Randy, Kathy, Auntie Lisa, and grandma thanks for always believing in me. This is for you.

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CHAPTER 1

Introduction

The use of natural enemies to aid in pest suppression has been the subject of an ocean of research, particularly in organically-managed agricultural systems where emphasis is often placed on non-chemical means of suppression(Greathead and Greathead 1992). While natural enemy control regimes can involve numerous strategies, the use of predatory arthropods as a means of direct and indirect control is often the focus (Symondson et al. 2002) However, while early predation studies imagined simplified linear "food chains", recent evidence has shown that generalist feeding is far more common in nature than previously assumed (Thompson et al. 2007). With this complexity in mind, understanding the intricacies of predator diets in field settings is increasingly crucial. In particular, generalist predators are often involved in conservation biological control with much research focused on understanding their role (Symondson et al. 2002, Birkhofer, Fließbach, et al. 2008, Roubinet et al. 2017). Molecular-based diet analysis has become an integral part of understanding the biological control potential of predators. Currently, the standard is using amplicon-based methods, wherein taxa-specific DNA regions are replicated hundreds to thousands of times before using existing databases to identify the specific organisms within the sample (Pompanon et al. 2012) The use of amplicons, which can amplify taxa-specific DNA regions, is a vast improvement over previous methods (King et al. 2008), allowing for the simultaneous detection of numerous diet items without complicated laboratory methods to create specific probes for each and every diet item of interest.

In addition, traditional agricultural study systems are limited in either geographic or taxonomic scope (Collins et al. 2002, Hooks and Johnson 2003, Landis 2017). While logistics are a natural limiting factor and such studies are able to be applied, but there are numerous examples of larger scale studies failing to replicate patterns seen in simplified systems (Koss and Snyder 2005, Blubaugh et al. 2021). This is especially true when studying generalist predators and in organic systems where biological connections are more important for biological control (Birkhofer, Bezemer, et al. 2008, Muneret et al. 2018). These limitations are at the heart of our inquiries into the role of *Pterostichus melanarius* as a biological control agent in organically managed farms in the Pacific Northwest (Busch et al. 2021). As a ground dwelling insect predator active observation of its diet in the field is difficult and as a generalist its impacts are likely to be more complex and happen at a more community level. The goal of this dissertation is to attempt to use both advances technology as well as taking a more system level approach to evaluating means of moderating biological control. These investigations will be encompassed in the following chapters.

Chapter 2

Are specialists really safer than generalists for classical biocontrol?

A review of the traits which determine the efficacy and risk of biological control agents to determine the role of predatory arthropods within biological control schemes.

Chapter 3

Using shotgun genomics to better determine *P. melanarius* diet in the field.

We compare shotgun metagenomics with traditional metabarcoding as a means of determining the diet breadth of the generalist predatory ground beetle *P. melanarius.* Beetles collected from organically managed farms had their gut content prepared via metabarcoding and shotgun metagenomics to detect diet items and compare their results. The intentions of this chapter are to:

1. Create a pipeline to take short reads created by shotgun sequencing and gather reliable identifications, specifically utilizing contig generation.
2. Determine biological control potential of *P. melanarius* based on detected diet items.

Chapter 4

Weedy Brassica fields encourage more diverse and abundant predatory insects

On the same farms from which we collected beetles for diet analysis, we will use collected on farm data and landscape data paired with insect populations determined by pitfall trapping, D-vac sampling, and visual surveys. The goal of this chapter is to:

1. Test local landscape factors for their impact on total insect community metrics as well as specific natural enemies and pests of interest in the brassica system.
2. Discuss the implications for conservation biological control schemes.

Chapter 5

Bird predation and landscape context shape arthropod communities on broccoli

On farm bird exclusion was paired with fecal diet analysis to better understand the top down impacts on what is generally assumed to be an insect structured biological controlled system. The goals of this chapter were to:

1. Evaluate the exclusion of birds on they yield of conventionally managed brassica plants.
2. Relate surrounding landscape context to the pest communities measured on farms.

3. Relate bird species diet data to predict their effectiveness as biological control or impact as intraguild predators in the system.

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Chapter 2

Are specialists really safer than generalists for classical biocontrol?[1]

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[1] J.M. Taylor and W. E. Snyder. 2021 BioControl 66 (1): 9-22. Reprinted here with permission of the publisher.

Abstract

Generalists rarely are considered for classical biocontrol because their broad feeding habits are expected to make non-target impacts inevitable. This assumes an increase in overall ecological risk with increasing number of feeding connections. With the goal of inspiring fresh consideration of the safety of exotic biocontrol agents for classical biocontrol, we present a selective review of the impacts of nine particularly-well-studied exotic natural enemies ranging from relative specialists to generalists. Surprisingly, non-target effects could be particularly strong for relative specialists that attacked just a few natives but were often widespread but weak for the broadest generalists. This appeared to reflect relatively strong apparent competition and density-dependence for the narrow feeders, versus broadly diffuse net effects for the broader feeders. Overall, we suggest a sole focus on specialists in classical biocontrol might be an unreliable means to reduce ecological risk. Additional research is needed to fully compare the net direct and indirect effects of generalists and specialists across food webs, following classical biocontrol releases

Keywords: Non-target effects, Risk assessment, Predator, Parasitoid, Non-native, Release

Introduction

Biological control was initiated, in its earliest days, as a strategy to manage invasive pests that had escaped their natural enemies when moving to new continents (Hajek and Eilenberg 2018). When successful, this approach has the potential to dramatically and sustainably reduce pest numbers and damage without the need for costly chemical or other pest controls (Hajek and Eilenberg 2018). However, broad-feeding generalists initially had a rocky history as introduced biological control agents. Problems began with the disastrous early introductions of generalist vertebrates such as the mongoose (*Herpestes javanicus*) in Hawaii and the cane toad (*Rhinella marina*) in Australia (Shine 2010), which caused devastating and wide-reaching ecological harm with little evidence that pest control was enhanced.

Things further worsened for generalists when evidence began to emerge that seemingly safer, smaller invertebrate generalists might also be having undesirable non-target effects. Examples include the decline of native Hawaiian puncture vine (*Tribulus cistoides*) by the herbivorous weevil *Microlarinus* spp. that was intended to control the vine's invasive congener *Tribulus terrestris* (Andres and Goeden 1971), and the extinction of several native Hawaiian land snails by the introduced predatory snail Euglandina rosea that was originally intended to control various pest gastropods (Civeyrel and Simberloff, 1996). In each of these cases, the generalists fed not only on the control targets but also on other naïve prey, which as part of depauperate island faunas had no co-evolutionary history with similar natural enemies. In the wake of these disasters the safety of all biological control efforts, not just those involving broad generalists, was called into question amid numerous calls for stricter host-specificity testing (e.g., De Clercq et al. 2011; Howarth 1983; Stiling and Simberloff 2000). The time and cost associated with this testing likely contributed to an overall decrease in classical biocontrol releases in North America and Europe, which have been responsible for a plurality of introductions (Fig. 2.1; Cock et al. 2016; Greathead and Greathead 1992, Babendreier et al 2006).

Arguably a central, although not often examined, tenet of linking ecological safety to specialization is the assumption that risk is greater with an increasing number of feeding links. With the goal of initiating discussion with the biocontrol community, here we pose the possibility that there could be reason to doubt this assumption (Fig. 2.2). Of course, an absolute specialist that feeds only on the control target, while being incapable of attacking any other prey/host species, cannot directly endanger any other species. But this does not mean that indirect effects of a strict specialist on natives will be weak (e.g., Callaway et al. 1999; Louda and Arnett 1999; Pearson and Callaway 2003). In addition, past the point of pure, single-species specialization, there is reason to think that variation in the magnitude of effects on particular native non-targets might not remain constant with increasing generality of feeding (Fig. 2.2). Indeed, evolutionary responses through time can lead to changes in the degree of host specificity following introduction (e.g., Tomasetto et al. 2017).

One possible complication is the common observation that natural enemies that simultaneously attack just a small handful of prey/host species are well-known to engage in "apparent competition" (sensu Holt 1977). Apparent competition occurs when predators supported by feeding on one prey species reach higher densities than they otherwise might, with these elevated predator densities leading to heightened predation on a second prey/host species (Holt 1977). This means that occasional "slip ups" in fully delineating a relatively specialized natural enemy's modest degree of polyphagy might yield relatively few, but particularly strong, non-target impacts (Fig. 2.2, 2.3). In contrast, the broadest generalists, by definition, feed on many different prey species and often across trophic levels (Polis et al. 1989). This means that non-target impacts will be widespread, sometimes remarkably so (e.g., Hurd et al. 2015; Hurd and Eisenberg 1990; Fig. 2.4). Yet, because the broad generalist enemy is not particularly dependent on, or able to dramatically benefit from, any single prey/host species, the abundant non-target effects might each individually be relatively weak (Fig. 2.2). Indeed, this type of broadly diffuse feeding relationships can dampen predator-prey oscillations to make exceptionally high densities of a particular pest species less likely (McCann et al. 1998).

Here, we provide an initial exploration of the relationship between a natural enemy species' degree of feeding specialization/generalization, and the number and strength of non-target impacts. We take advantage of detailed ecological work for nine particularly well-studied introduced insects. In our selective review, because very broad generalists are usually excluded from classical biocontrol programs, we had to rely on several case studies where the natural enemy was accidentally rather than intentionally released. So, our goal is not to examine or critique previous biocontrol releases; rather, we intend to initiate a broader discussion of the relationship between specialization and ecological safety using the most complete case studies available in the literature. These ranged from the relative specialist weevil *Rhinocyllus conicus* (Coleoptera: Curclionidae), leaf beetle *Diorhabda elongata* (Coleoptera: Chrysomelidae), and parasitoids *Cotesia rubecula* and *Cotesia glomeratea* (Hymonoptera: Eulophidae); to the intermediate polyphagous beetles *Coccinella septempunctata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) and the tachinid fly *Compsiluara concinnata* (Diptera: Tachinidae); to the broadly polyphagous generalist praying mantid *Tenodera sinensis* (Mantodea: Mantidae) and ground beetle *Pterostichus melanarius* (Coleoptera: Carabidae). We use these case studies to examine how host-specificity, or lack thereof, impacted the severity of their non-target effects through both direct and indirect channels.

Case studies

Too hot? Relatively narrow specialists

The weevil *Rhinocyllus conicus*

Rhinocyllus conicus was introduced to North America in the late 1960s to early 1990s to control *Carduus nutans* and other invasive European thistles (Kok 2001; Kok and Surles 1975; Turner et al. 1987). Like many weed biocontrol agents, R. conicus underwent host-specificity testing before release, which included documenting an apparent preference for attacking the target weed

C. nutans and low usage and larval survival on non-target thistles (Zwölfer and Harris 1984). In addition to this perceived narrow host range, it was also hypothesized that *R. conicus* would be outcompeted by native seed predators on any native thistles because the weevil is relatively uncompetitive with guildmates in its home range (Zwölfer 1979). These initial studies laid the groundwork for what seemed like the perfect scenario of high target impacts and little native spillover, to the far left of Fig. 2.2. However, while reducing seed count of *C. nutans* by over 90%, *R. conicus* also attacked the rare Platte thistle, *Cirsium canescens* (Louda et al. 2003; Wiggins et al. 2010). This non-target utilization occurred despite the preference *R. conicus* still exhibited for its control target, *C. nutans* (Arnett and Louda 2002; Gassmann and Louda 2001). Apparently, abundant *R. conicus* emerging from the biocontrol target "spill over" to also attack the less preferred, at-risk native plant (Blitzer et al. 2012); that is, the classic apparent competition scenario (Fig. 2.3a). Overall, this forms an example of the particular risk that relative specialistscan pose to non-targets when they spill 114 over from large populations on the control target, shown as the peak on the left side of our specialism-generalism risk model (Fig. 2.2).

The parasitoid wasps Cotesia rubecula and Cotesia glomerata

Comparing the closely related parasitoid wasps *C. rubecula* and *C. glomerata* further elucidates the line that relatively specialized control agents can straddle, from relatively pure focus on a target pest to apparent competition, spillover, and harm to sensitive natives. While not part of formal biocontrol programs, the *Cotesia* spp. either entered North America accidentally or as part of amateur control efforts (Clausen 1978; Scudder 1889). Of the two, *C. rubecula* is considered to be a nearly complete specialist on the crucifer pest *Pieris rapae*. In contrast, its congener *C. glomerata* attacks both *P. rapae* and related, native non-target *Pieris* spp. (Brodeur et al. 1996; Krombein et al. 1979). In turn, *C. glomerata*'s relative polyphagy has been linked to the shrinking ranges of the sensitive native *Pieris napi oleracea* (Van Driesche et al. 2004). This is because, in areas where the parasitoid occurs alongside both *P. rapae* and *P. n. oleracea*, *C. glomerata* parasitoids emerging from the relatively abundant *P. rapae* spill over to attack *P. n. oleracea*; that is, yet another example of apparent competition that is harmful to a native non target (Fig. 3a; Benson et al.2003a; Benson et al. 2003b). In summary, the two *Cotesia* species appear on opposite sides of the inflexion point between nearly-pure specialization on a pest without substantial spillover to non-targets in the case of *C. rubecula*, versus the slight increase in generalism leading to spillover and harmful apparent competition for *C. glomerata* (Fig. 2.2, 2.3a; Cameron and Walker 2002; Van Driesche et al. 2003).

The herbivorous beetle Diorhabda elongata

The herbivorous leaf beetle *D. elongata* was introduced into the western United States to control non-native saltcedars (*Tamarix* spp.), which invade and come to dominate riparian areas. As a relatively recent biocontrol introduction, host specificity for the beetle was a high priority, as was its destructive potential against the control target (DeLoach et al. 2003). Host specificity tests evaluated *D. elongata* preference and performance on non-target shrubs. Though usage of *Frankensia*, a close relative of *Tamarix*, was as high as 15% in some studies, field tests yielded minimal evidence of risk for defoliation in a more "natural" setting for native *Frankensia* or *Tamarix aphylla* plants (Dudley and Kazmer 2005; Herr et al. 2009; Lewis et al. 2003). With little apparent risk, *D. elongata* was released in the early 2000s. Follow-up studies have found little evidence that *D. elongata* has adapted to utilize natives more than initial host testing had predicted (Pratt et al. 2019). In terms of our theoretical model, then, *D. elongata* would appear to be a prime example of a nearly-pure specialist, with near-total focus on the control target that is thought to be associated with no measurable non-target effects (far left side of Fig. 2.2).

However, there may be reason for caution. Most significantly, the rapid removal of *Tamarix* from its invaded range may harm endangered bird species that rely on these trees for nesting sites (Fig. 2.3b; Bean and Dudley, 2018; Paxton et al., 2011; Sogge et al., 2008). This is a classic example of a strong indirect effect on native communities that can lead to harmful non-target effects even when direct harm to non-targets is relatively weak. It must be noted that the effectiveness of this classical biological control effort relative to chemical or other weed control methods also, perhaps somewhat ironically, underlies the strength of its unintended indirect effect on the birds. Furthermore, several ecotypes of *D. elongata* have been released, and hybridization between these "strains" could lower 160 host-specificity in the future (Bitume et al. 2017). There also is some initial evidence that the beetles' host fidelity may be reduced after establishment when compared to a laboratory reared cohort (Thomas et al. 2010). This sets up the possibility that apparent competition could emerge, if large populations of beetles on invasive *Tamarix* begin spilling over to increasingly attack its native relatives. That is, the situation could move towards the "increased risk hump" on the left side of our conceptual model, reflecting increasingly-strong apparent competition (Fig. 2.2).

Too cold? Broad generalists

The praying mantid Tenodera sinensis

The praying mantis *T. sinensis*, native to Asia, does not appear to have been intentionally introduced into North America; rather, it may have arrived in egg sacs attached to nursery stock (Hurd et al. 2015). Regardless, this mantis has been the subject of extensive study, including a series of manipulative field studies in the old-field early successional plant communities where they occur, that provide detailed insight into ecological effects in the invaded range (e.g., Hurd and Eisenberg1990; Hurd et al. 2015). This has revealed that, while the mantid appears to exert a bewildering number and diversity of direct and indirect effects on other community members, no single link appears to be particularly strong. For example, comparison of large field plots with versus without *T. sinensis* populations revealed that these mantids weakly, directly suppressed numbers of larger herbivorous insects (Hurd and Eisenberg 1990). However, these predators also exerted complex effects on smaller herbivores through a balance between direct predation and release from predation by wolf spiders and other meso-predators (Hurd and Eisenberg 1990; Moran and Hurd 1998; Hurd et al. 2015); the mantids appear to drive wolf spiders from areas they inhabit as the spiders emigrate to avoid intraguild predation (Moran et al. 1996). Altogether, these varying effects on different predaceous and herbivorous prey species, through both direct and indirect channels and resulting both from actual predation and *T. sinensis*-induced changes in behavior, dampens any resulting trophic cascade impacting plant biomass (Hurd and Eisenberg 1990; Moran and Hurd 1998). So, from the standpoint of non-target effects, the news is generally good because while *T. sinensis* is impacting many different native species, these numerous links appear to be generally weak (Fig. 2.4a). From the standpoint of herbivore suppression, however, the many weak links do not sum to yield a dramatic increase in plant biomass (Fig. 2.4a). Were this a biocontrol system, then, *T. sinensis* is not substantially harming native non-targets but it also is not substantially strengthening natural control of herbivores to the benefit of plant yield (i.e., this case study equates to the far right of Fig. 2.2).

The ground beetle Pterostichus melanarius

The ground beetle *Pterostichus melanarius* is another broad generalist, native to Europe but accidentally introduced to North America in ship ballast, whose ecological impact bears many similarities to that of *T. sinensis* (Snyder and Evans 2006). In both the native and introduced range, *P. melanarius* appears quite tolerant of human disturbance and often is abundant in agricultural fields (Busch 2016). The beetles are remarkably polyphagous, feeding on a great diversity of herbivorous, predacious, and detritivorous arthropods and other prey species (e.g., Hagley et al. 1982). As with *T. sinensis*, this in turn leads to a diversity of direct and indirect effects that impact pest suppression. For example, in the northwestern U.S., *P. melanarius* feeds on eggs and larvae of *Delia* spp. flies that otherwise can heavily damage the roots of crucifer crops (Prasad and Snyder 2006). However, *P. melanarius* also triggers other ground and rove beetle species to forage less often, apparently as the smaller beetles seek to avoid intraguild predation, such that *P. melanarius*'s direct feeding does not benefit the control of fly pests (Prasad and Snyder 2006). In turn, attempts to augment biological control by ground beetles by providing them with weedy refuges in or near agricultural fields, yields a complex mix of benefits and harms. The refuges benefit both *P. melanarius* and the smaller native predaceous beetles, strengthening control of herbivorous root-pest insects by the smaller beetles alongside disrupted foraging of these smaller predators in the presence of *P. melanarius* (unpublished data). On the whole, then, all of these many weaker feeding connections yield no clear net contribution towards, or disruption of, pest insects by *P. melanarius*. Furthermore, *P. melanarius* appears only to be common in disturbed habitats, and has not been found to disrupt native carabid communities in forests (Niemelä and Spence 1991; but see Magura et al. 2010). Even in more urban settings, where *P. melanarius* is abundant, native populations of carabids do not seem to suffer as a result (Niemelä and Kotze 2009). It may be that *P. melanarius* is primarily exploiting habitats to which native carabids are ill-suited, leaving a natural refuge for the natives in less-disturbed areas.

Just right? Intermediate generalists

The lady beetles Harmonia axyridis and Coccinella septempunctata

Some readers might be surprised by the labeling of these two generalists as "just right" for classical biocontrol. After all, *H. axyridis* and *C. septempunctata* are arguably two of the most maligned non-native generalist predators where they have been released or have invaded. This largely stems from perceived harm the two lady beetles have done to native coccinellids, which is alleged to result, at least in part, from intraguild predation of natives by the non-natives (e.g., Brown and Roy 2018; Sloggett 2017). It is clear that the arrival of one or both of these lady beetles to new habitats has at least roughly correlated with fewer native lady beetles found foraging in agricultural fields (e.g., Alyokhin and Sewell 2004). But recent years have seen growing skepticism that fewer natives in cropping fields reflects a true regional decline outside of these highly-managed systems (e.g., Riddick 2017; Sloggett 2017). Some particularly elegant work was reported by Evans (2004), who recorded the near-disappearance of native lady beetles from Utah, U.S.A., alfalfa (*Medicago sativa*) fields following the arrival of *C. septempunctata*. This seemed consistent with intraguild predation of the natives by *C. septempunctata* leading to native declines, although numbers of pea aphids (*Acyrthosiphon pisum*) in alfalfa also decreased, apparently due to relatively effective predation by *C. septempunctata*, such that resource competition could be at play. And indeed, when pea aphid densities were experimentally restored to pre-invasion levels, native lady beetles once again returned to alfalfa (Evans 2004). This suggests that native lady beetles exploited the dense aphid resources that agricultural fields provided when available, but when aphid densities dropped the natives simply remained within the natural habitats they presumably had inhabited before the arrival of agriculture (Evans 2004). So, there is evidence in this case that *C. septempunctata* is having the desired effect, driving down numbers of pest aphids in agricultural fields, while native lady beetles find refuge in more natural habitats.

Something similar may be at work for *H. axyridis*. Arrival of this non-native predator has been associated with declines in several previously-common aphid species in agricultural habitats (e.g., Riddick 2017), consistent with *H. axyridis* strengthening aphid biological control. In the midwestern U.S., years with relatively high densities of *H. axyridis* correlate with relatively few collections of ladybird beetles from in or near agricultural fields (Bahlai et al. 2015; Diepenbrock et al. 2016). Yet, native lady beetles remain relatively common in more natural habitats, consistent with aphid suppression in agricultural fields due to *H. axyridis* predation driving natives back into natural habitats they utilized before the broad introduction of agriculture (Bahlai et al. 2015; Diepenbrock et al. 2016). Likewise, in Europe, it appears that the rise of *H. axyridis* in urban habitats, where pollution-stressed trees experience relatively large aphid outbreaks, may simply be returning native *Adalia* lady beetles to natural habitats they had previously occupied (Viglášová et al. 2017); apparent declines in *Adalia* had previously been attributed to intraguild predation by *H. axyridis* (e.g., Brown and Roy 2018). So, while there is little doubt that intraguild predation of natives by *H. axyridis* is common under laboratory conditions and also occurs in the field (e.g., Brown et al. 2015; Ware et al. 2009), it seems that depletion of prey resources in human-managed habitats is the main mechanism through which the non-native beetles are displacing natives (Alyokhin and Sewell 2004; Kindlmann et al. 2011) (Fig. 2.5). Again, of course, it is just this sort of depression of pest aphids in agricultural fields that is the goal of biological control. Both the relative segregation of *H. axyridis* into trees rather than herbaceous crops (Honek et al. 2019; Masetti et al. 2018), and the gradual accumulation of parasitoids attacking *H. axyridis* in the introduced range (e.g., Ceryngier et al. 2018) may further diminish any risk that these beetles pose to natives. More generally, both *C. septempunctata* and *H. axyridis* may fall into the "just right" place on our risk curve (Fig. 2), being sufficientlygeneralist to take advantage of alternative 275 prey when pest aphids are not abundant, but specific (and mobile) enough to be drawn to pest aphid outbreaks when the occur (e.g., Bannerman et al. 2018). As a caveat is important to note, however, that while both *C. septempunctata* and *H. axyridis* are sometimes found in natural habitats alongside native lady beetles (e.g., Diepenbrock and Finke 2013; Grez et al. 2017) relatively little is known about their interactions there such that harm to the natives may be occurring that is not documented.

The tachinid fly Compsilura concinnata

In some respects, the tachinid fly *C. concinnata* is considered a classic example of why generalists should not be considered for classical biological control (Elkinton and Boettner 2012). In North America, the fly was first released in the early 1900s in an attempt to control two European invasives, the gypsy moth (*Lymantria dispar*) and the browntail moth (*Euproctis chrysorrhoea*), that were devastating hardwood forests (Elkinton and Boettner 2012). As these introductions were early in the 20th century, formal host-specificity testing was not conducted but the fly was known to attack > 200 species of lepidoptera and symphyta (Elkinton and Boettner 2012). Unfortunately, while the gypsy moths that were the primary target for control are univoltine and is only in the vulnerable larval stage at the beginning of the summer, *C. concinnata* is multivoltine and so must switch to attacking non-target hosts later in the growing season (Webber and Schaffner 1926; Weseloh 1982). Because native saturniid moth larvae are among the only vulnerable hosts later in the year, in turn this means that *C. concinnata* has spilled over to devastate these large, charismatic natives (Boettner et al. 2000; Kellogg et al. 2003). So, here again, this is a classic case of apparent competition, albeit with the control target and the most-impacted non-natives occurring 298 separate in time (Elkinton and Boettner 2012). This case study presents a cautionary note, then, the moderate generalism will not always generate the "right" mix of strong effects on the target and relatively weak non-target harm. Further work will be needed to see whether the details of how non-target harm operates in this system – continuing relatively high densities of pests after the biocontrol introduction, relatively brief seasonal susceptibility of the control targets that is staggered with when the natives are susceptible – are broadly common in other systems.

Discussion

The need to document extreme specialization as the key criterion indicating safety of a natural enemy being considered for release, has greatly slowed progress in classical biological control (Cock et al. 2016; Follett et al. 2000; Messing and Wright 2006). After all, host-range testing is expensive, time consuming, and difficult to conduct under quarantine conditions (Messing 2009; Messing and Brodeur 2018). If this approach yielded the only accurate measure of likely ecological risk associated with enemy release, few would quibble with the cost and delays. Here, we considered a few particularly well-studied natural enemies in their non-native ranges, chosen to represent a range of feeding habits from relatively specialized to relatively generalized. Intriguingly, this selective review suggests no clear relationship between specialization and safety. Rather, existing work has found non-target effects across ranges of specialization, varying in the number and strength of feeding relationships, and of effects operating through direct and indirect channels, rather than any simple increase in risk as generality increased.

We contrasted the results from our selective review of the few most-detailed ecological case studies that were available, with a simple graphical model of the relationship between degree of specialism/generalism and impacts on target and non-target prey (Fig. 2.2). We emphasize that, even if the case studies perfectly fell along the line predicted by the null model, 9 case studies would be far too few to confidently reject or accept the model's validity. We predicted that apparent competition might quickly amplify any non-target effects of relative specialists, as natural enemy densities bolstered by the control target allowed the agent to spill over to harm even relatively less-preferred native non-targets. Indeed, there may be some support for this possibility, as relatively specialized natural enemies that attack targets as different as exotic thistles and butterflies showed signs of apparent competition that substantially harmed natives. We predicted that these non-target harms would then grow more diverse, yet less impactful, with increasingly general feeding habits (Fig. 2.2). Here again, particular case studies provided evidence this might be the case, as several notorious lady beetle introductions appear, after careful consideration of recent findings, to be improving aphid biocontrol while displacing native lady beetles from managed habitats due to resource competition, rather than devouring them into extinction. However, it is important to note that a third case study, that of the tachinid fly *C. concinnata*, provided an example of substantial non-target harm from a moderate generalist. Finally, at the most extreme, while the broadest generalists by definition feed on many different non-target prey, these attacks are so diffuse that substantial harm is unlikely to be sufficiently widespread and consistent to lead to major declines in any particular prey species. Of course, alongside this, impacts on pests will likely be so diffuse as to make biological control introductions of the broadest generalists unlikely to measurably improve pest control.

An additional consideration that emerged from our selective review is that the strength of indirect effects that exert impacts on non-natives may be just as strong as those resulting from the direct effects that host-range-testing seeks to minimize (Moore 1989; Pearson et al. 2000). This reflects a broader understanding in ecology that indirect effects are a key force that structures communities (Berlow 1999). We saw evidence for a relationship between degree of generalism, and the extent and strength of indirect effects impacting non-targets, similar to that seen for the direct non-target effects discussed just above. When a natural enemy is highly specialized, and focuses its attacks on a single target prey/host, this can lead to sharp reductions in the target that have strong indirect effects on other native community members. This possibility has been most-discussed in the case of biocontrol of *Tamarix*, which serve as important nesting habitat for some rare riparian birds (Hultine et al. 2010; Sogge et al. 2008). But we would expect similarly-strong indirect effects anytime an invasive species is quickly and effectively pulled from the invasive range. Of course, the harm of these indirect effects will likely diminish through time, and native species move in to fill the niche(s) the now-controlled exotic once occupied. The broadest generalists might initiate a bewildering diversity of indirect effects, considering the large number of non-target prey they attack, but, because each direct link is relatively weak, we would not predict any single indirect effect to be as strong as those generally exerted by specialists.

Conservation biological control, which does not need to consider the non-target risks associated with classical biological control efforts, has often focused on bolstering generalist predator populations to strengthen natural pest control (e.g., Hessel 2013). This work provides a roadmap for the situations where addition of a generalist natural enemy might markedly strengthen pest suppression. First, generalists often form a “first line of defense” as pests colonize a field, because they are able to persist on non-target, alternative prey and are therefore present and active when initial pest colonization occurs and forming a “natural enemy ravine”(Symondson et al. 2000, Southwood and Comins 1976). However, this works best when generalists readily switch to attacking the control target when it becomes available (e.g., Settle et al. 1996). This important host switching behavior might be predicted with host-range testing before a release is made, such that this exercise might still be useful for generalists. We must also note that there could be cases where relatively rare (or slowly reproducing, etc.) native prey are the only option for a particular generalist species in some locations or times of year, such that non-target predation that makes up a relatively small portion of a generalist’s overall diet could nonetheless yield substantial harm to the native prey. Indeed, this is precisely the reason that *C. concinnata* is so harmful to native moths in the eastern US. Second, while generalists may not exert a density-dependent increase in impact on a pest that is consistent with prey suppression, generalists can dampen the rate of pest increase and complement the density-dependent impacts of specialists (e.g., Snyder and Ives 2003). Third, generalists are particularly useful control agents in crops that face complexes of different pest species, because they can move from attacking one prey species to another as different pests become active or problematic throughout the season (e.g., Neuenschwander et al. 1975). Here again, how host switching impacts biocontrol will depend in part on relative preferences of a generalist for target versus non-target prey species. Finally, generalist predators that occupy different spatiotemporal feeding niches are most likely to complement one another, without co-occurring in space and time such that intraguild predation might be tempting (Snyder 2019). Together, these findings suggest when the addition of a new generalist to a guild of biocontrol agents might be particularly valuable to natural pest control. The case studies provided here suggest that a preference for human modified over natural habitats, and an ability to aggregate at sites where target pests are reaching outbreak levels, are additional traits of some generalists that would be likely to strengthen their roles in classical biological control without enhancing risk to non-target natives.

Future directions

Altogether, our selective review suggests limitations in equating specialization with ecological safety. We propose that the current view relies too heavily on consideration of direct effects on non-targets. When instead setting these non-target effects within a real-world context where apparent competition and indirect effects are also considered, the relationship between degree of polyphagy and ecological safety becomes rather muddled. Looking from the community perspective, a "Goldilocks" situation might be best, with moderate generalists best able to persist among non-pest prey when needed, yet numerically respond to increases in pest densities when this is needed. That is, specialists might be a bit "too hot" and broad generalists "too cold", with intermediate generalists "just right". We also highlight the need to separately consider effects of introduced generalists in the unique context of agricultural fields, versus in natural settings where non-targets might seek refuge.

We close by suggesting a few next steps that might be particularly fruitful areas for future exploration:

1. Expanded meta-analysis of the specialism-risk relationship. Here, we focused on a few carefully chosen case studies where ecological studies were relatively abundant. Yet, this falls short of delineating the relationship between specialism and ecological risk across the full diversity of biological control agents that have been released worldwide. It is possible that a more comprehensive synthesis would find a general increase in substantial non-target harm with increasing generalism that our hand-picked small sample of case studies could not detect, and would be a worthy target of a true synthetic meta-analysis across all available case studies. In turn, a broader meta-analysis of this type would allow a formal test of the relationship between generalism and impacts proposed here (Fig. 2.2).

2. How best to determine risk? While we suggest that specialized feeding, on its own, does not greatly predict degree of risk, we do not deny that host-specificity is and should remain an important part of risk assessments. However, the specificity of the proposed agent should be weighed against the expected pressure on the non-target groups effected. In addition, certain groups such as specialist herbivores and parasitoids may merit closer scrutiny than predatory agents since while the former groups are likely to have greater host-specificity, their life histories usually allow for rapid population growth and correspondingly large ecological effects. Likewise, indirect effects can form a key component of ecological risk and are worthy of greater attention.

3. Increased post release studies. While we do make the case that the damage caused by generalist biocontrol agents, and generalist predators in particular, are overstated, there is still a knowledge gap when it comes to non-target effects of most introductions. In the future more focus should be placed on monitoring likely non-target hosts/prey. While this is likely to be difficult and potentially expensive, it would be crucial work in allowing adequate risk assessments in the future.

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Figures

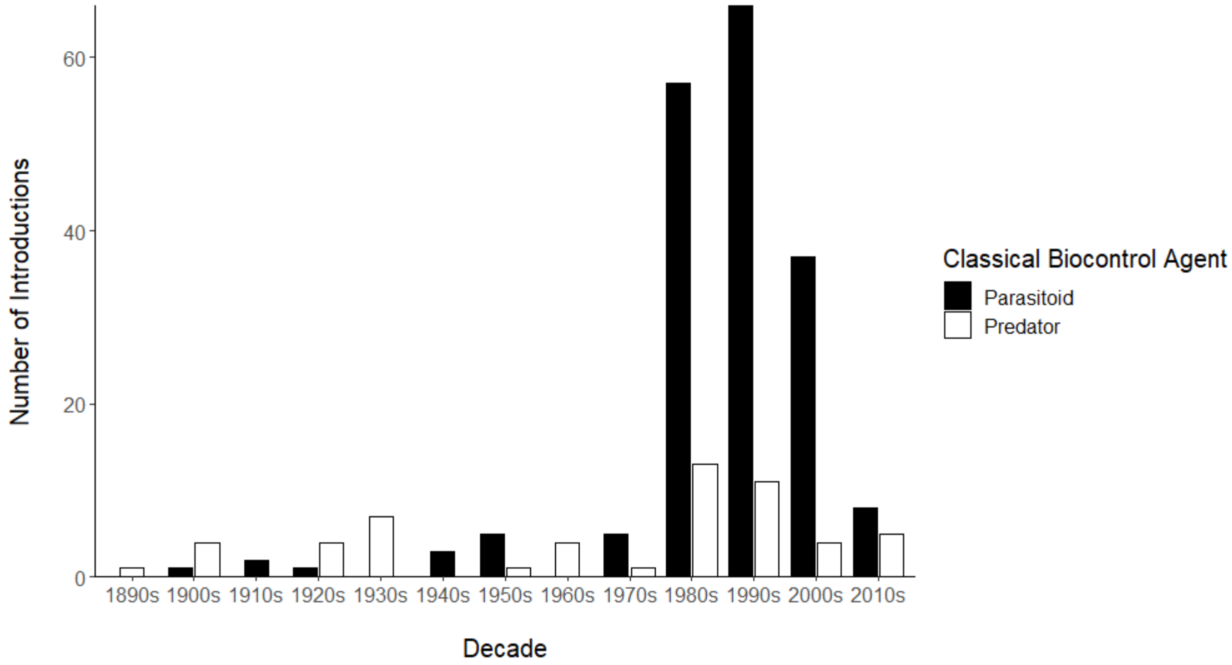

Figure 2.1 Number of exotic predator and parasitoid biocontrol agents released in North American and US territories and Europe, each decade from the 1890 s to 2010s. For species introduced several times, only the initial release is displayed. Date from van Driesche et al. (2018) and Gerber and Schaffner (2016)

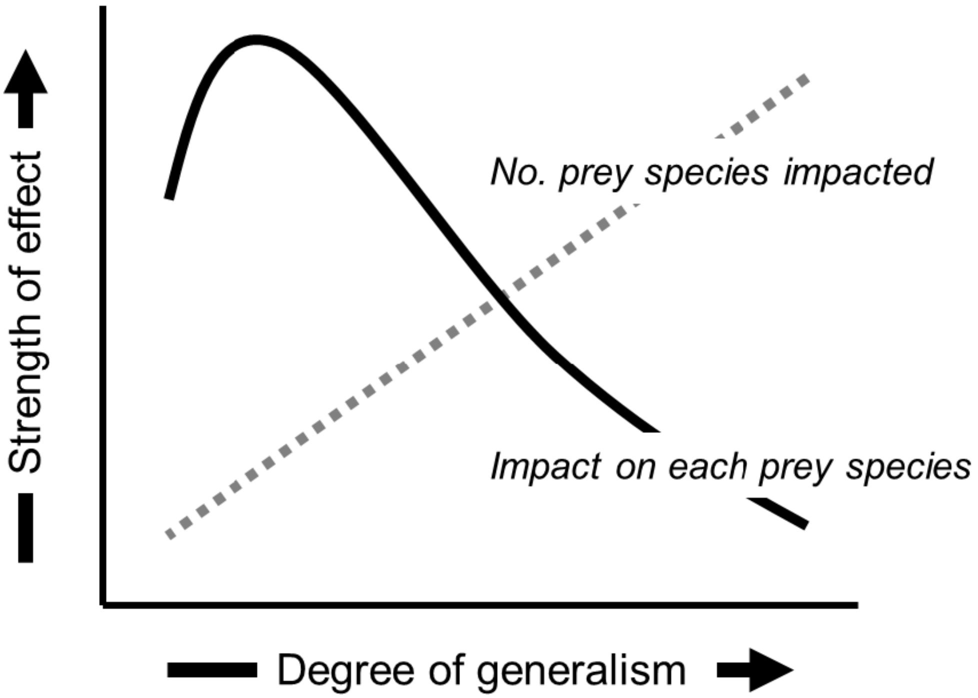

Figure 2.2 A simple graphical model of one possible relationship between degree of specialization by a natural enemy released for classical biological control and the magnitude of resulting effect, for the number of prey species impacted (dashed line) and the impact on each prey species (solid line)

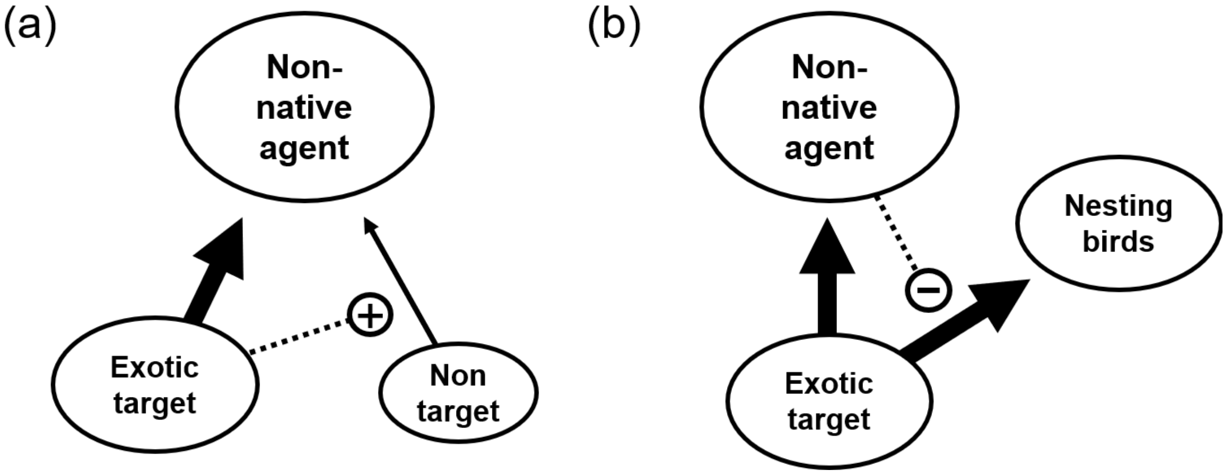

Figure 2.3 Examples of theoretical non-target effects**. a** A relatively specialized agent that strongly impacts the control target, with relatively little feeding on a non-target, might nonetheless exert considerable harm through “apparent competition” when agents reach large numbers while feeding on the target and spillover to attack the native. **b** When a control target provides habitat or supplemental food resources for a native species, the agent can indirectly harm the native by removing these resources. Solid lines indicate direct interactions and point from resource to consumer. Dashed lines indicate indirect effects, with those ending in “+” indicating an indirect effect that enhances the interaction and those ending in a “-“ indicating an indirect effect that weakens the interaction. Thicker lines indicate relatively stronger interactions.

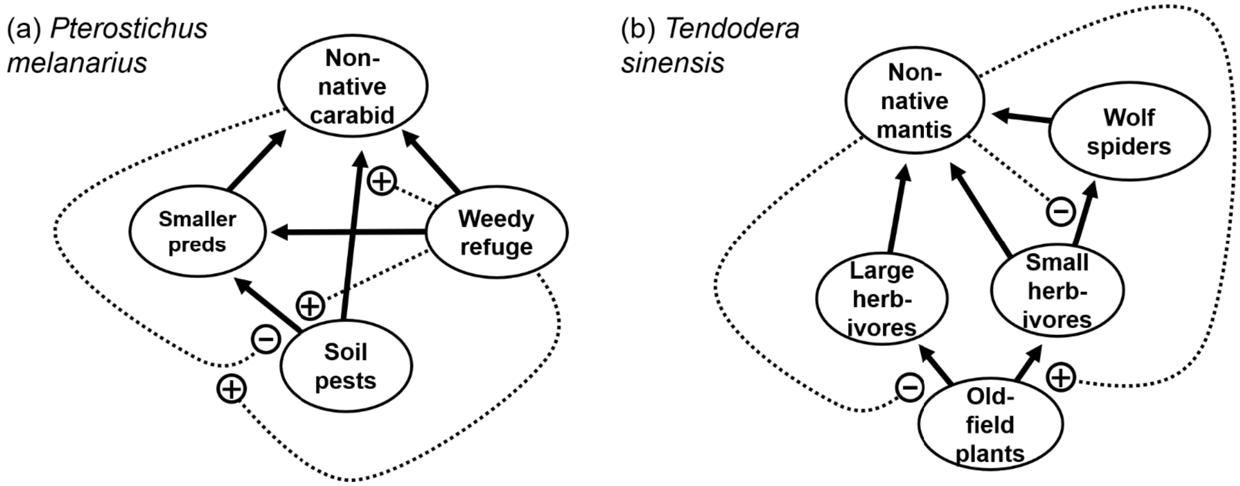

Figure 2.4 In North America, interactions between two non-native, broadly generalist predators and their communities. **a** The ground beetle Pterostichus melanarius feeds both on pests, strengthening biological control, and on smaller generalist predators, weakening biological control. The net impact of these counterbalancing positive and negative effects is further mediated by weedy refuge plantings that can benefit both the smaller native predators and the

intraguild predator P. melanarius. **b** Likewise, the praying mantis Tenodera sinensis feeds directly on some herbivores while also attacking wolf spiders and other smaller generalist predators. Wolf spiders will leave areas where mantids are present, further weakening the spiders' impacts on herbivores. This leads the mantid to exert a complex mix of indirect benefit to plants through direct suppression of herbivores that is largely counterbalanced by indirect harm to plants mediated by intraguild predation. Solid lines indicate direct interactions and point from resource to consumer. Dashed lines indicate indirect effects, with those ending in "+" indicating an indirect effect that enhances the interaction and those ending in "–" indicating an indirect effect that weakens the interaction

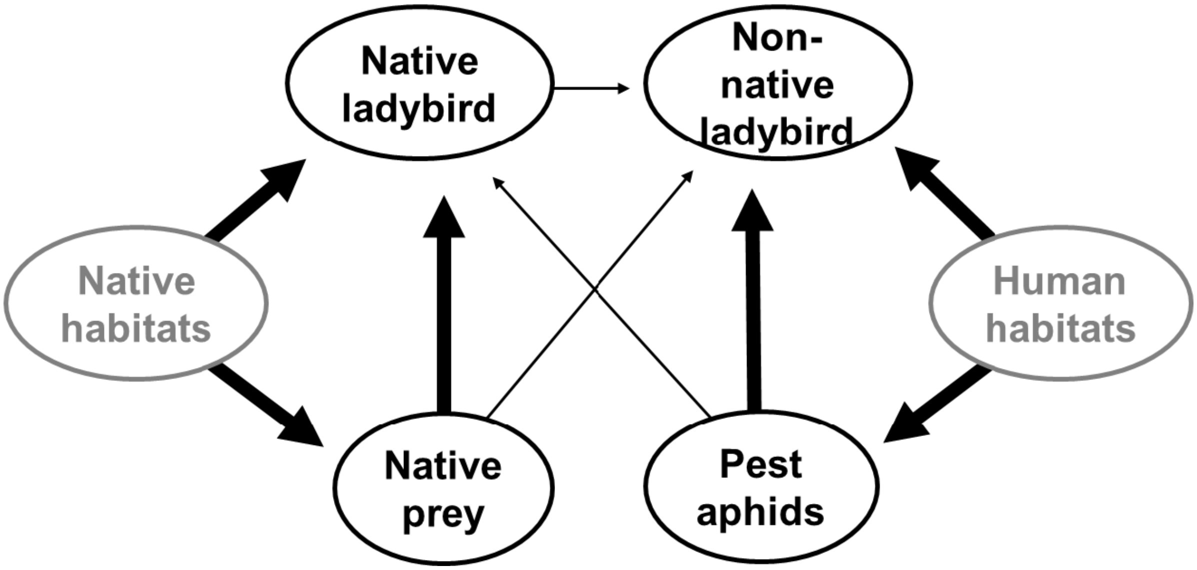

Figure 2. 5. Relationship between native and non-native conspecifics. The non-native ladybird beetles Coccinella septempunctata and Harmonia axyridis appear to concentrate their foraging in agricultural and other human-modified habitats, whereas native ladybirds may focus their foraging in less-disturbed habitats. This can lead to improvement of biological control in

cropping fields where the non-natives best tolerate disturbance and exploit pest outbreaks, while natives find refuge in the natural habitats where they presumably existed before conversion of land to agriculture. Solid lines indicate direct interactions and point from resource to consumer. Thicker lines indicate relatively stronger interactions

Chapter 3

Shotgun Metagenomics elucidates the diet breadth of *Pterostichus melanarius* (Coleoptera: Carabidae)[2]

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[2] Taylor, J.M., Wilson-Rankin, E. and Snyder, W. E. To be submitted to Biological Control as a research article

Introduction

Understanding the trophic relationships between members of a system is integral to ecology (Linderman 1942, Cook 1977). However, direct observations of predation events are difficult, due to their rarity; this is compounded when studying small predatory insects (Grieshop et al. 2012, Mansfield and Hagler 2016). The advent of molecular diet analysis, first with Sanger sequencing and more recently with next generation sequencing (NGS), has made detecting predation "after the fact" not only possible but increasingly efficient (Pompanon et al. 2012). However, while the current standard of metabarcoding allows for detecting even small traces of ingested DNA, the technique is not without drawbacks. One major concern is the technical difficulty of developing blocking primers to avoid the replication of the predators' DNA from crowding out that of its prey during the amplification process. Countering this usually requires creating a blocking primer in order to prevent the predator DNA from being replicated at all (Piñol et al. 2015, Vestheim and Jarman 2008). Another means to avoid this would be to use primers which are specific to taxa that are different from the host predator. Obviously, this would limit the systems that could be examined. In addition, for generalists, a single primer may not encompass all possible prey items, in which case a multiplex primer needs to be designed and tested to cover the breadth of potential prey items. This process is often quite technical can be prohibitively complex and there are limits to how widely this technique can cast its net (King et al. 2008a, King et al. 2008b). Compounding this issue is the fact that primers focus on very specific gene regions (cox1, rRNA, etc.) and these regions have not been outlined or have had primers created for them even when genomic work has been conducted on them (Kress and Erickson 2008, Rubinoff et al. 2006).

Fortunately, the increasing throughput from modern sequencers has allowed other options to become increasingly viable. Shotgun metagenomics involves shearing DNA and then sequencing it all independently, followed by more bioinformatic processing to identify the fragments. This method makes diet analysis possible even when the taxanomic breadth of diet items is unknown. It also circumvents the need for a blocking primer or constructing a primer multiplex (Sharpton 2014, Quince et al 2017). However, while the high throughput from newer sequencing technologies produces millions of reads, the length of these reads can be limited, especially when using the highest throughput platforms (Kircher and Kelso 2010). In which case, it can be difficult to align reads against a database, especially since there is no target region as is the case with metabarcoding. Consequentially, the downstream bioinformatics becomes increasingly important, and the sensitivity depends on the completeness of the DNA reference database (Normand and Yanai 2013). Another benefit of the technique is the possibility of also detecting internal parasites, which can be indicative of predation on obligate hosts (Paula et al. 2016). In addition, sufficient sequencing depth could also allow for analysis of the predator gut microbiome as well, which would increase the number of questions that could be asked from a single sequencing run.

Our efforts here are designed to examine the benefits and feasibility of using shotgun metagenomics for examining the on-farm diet of a generalist arthropod predator common in Pacific Northwest farming systems, *Pterostichus melanarius* Linnaeus (Coleoptera: Carabidae). *P. melanarius* has been shown to have a vast potential diet and have been shown to exhibit feeding on both agricultural pests and plant matter, while also being important intraguild predation on other natural enemy species (Hagley et al. 1982, Johnson and Cameron 1969, Firlej et al. 2013, Hatteland et al 2010). We collected beetles from farms employing organic practices across the U.S. states of Oregon, California and Washington. On-farm conditions were collected in tandem with beetles in order to assess how such factors might impact the diet of *P. melanarius*. To compare shotgun metagenomics to metabarcoding, we prepared DNA extracted from each beetle gut for both methods. We also compared methods for generating contigs from sequencing reads to form a more accurate representation of diet composition. Using shotgun metagenomics, we were able to identify several pest taxa, some of which were novel, for *P. melanarius*. While metabarcoding was much simpler from a metabarcoding sense, the identifications were, predictably, concentrated on a much narrower scope of taxa than shotgun metagenomics. While we detected no pattern of local habitat on the diet composition of *P. melanarius*, our efforts create a framework of such examinations in other predators and in other systems. Such examinations would greatly benefit conservation biological control schemes.

Methods.

Site Descriptions

Across 2 years (2016–2017), we conducted surveys to collect *P. melanarius* and collect farm data in Washington, Oregon, and California, USA, previously described in Smith et al. (2020, 2022) (Figure 3.1). Farms grew a range of crops including cereals (e.g., corn, wheat, barley), vegetables and melons (e.g., brassicas, leafy vegetables), fruits and nuts (e.g., citrus fruits, grapes, berries, walnuts), oilseed crops (e.g., olives, sunflower), roots (e.g., potatoes), spice crops (e.g., chilies, peppers, fennel), beverage crops (e.g., tea), medicinal crops, commercial flowers, and grasses and fodder crops, among others. All farms grew brassica crops including broccoli and/or kale. Farms in the study were generally small scale (mean ± SE = 25.3 ± 47.2 ha; range: 0.44–272.2 ha).

Cover Quantification

Percent cover was quantified on each farm by taking 10 equally spaced picture transects with a 1 x 1 meter Daubenmire frame. Each picture transect was broken down into a 100 square grid and the percentage of non-bare ground was calculated. The % cover from each transect was then averaged for each farm.

Insect collections

Pitfalls: 5 pitfalls were placed every 2 meters or equally spaced in fields less than 10 meters across. Pitfall transect was also placed as near to the center line of the field as was possible in order to limit edge effects. Pitfalls were set during the evening (from 17:00-19:00) and collected the following morning. To avoid contamination from regurgitates, pitfalls were "dry" and to avoid predation events with the traps they were partially filled with debris to allow for refuge (citation). Pitfalls were placed on dry ice to kill beetles and to preserve them for later dissections.

Molecular Methods

Collected *P. melanarius* had their forecrops dissected out and DNA was extracted using the DNEasy blood and tissue kit. The protocol was modified by heating elution buffer to 50 degrees C and using only 100 micorliters of buffer to increase concentration. Samples were quantified by Quibit.

PCR-free preparation

DNA samples were sent to the UGA Genomics core for PCR-free library preparation and sequencing. Samples were run on an Illumina NextSeq 2000 on a 2 x 150bp flow cell. For Metabarcoding sequencing, sample was then PCR amplified for arthropod specific CO1 regions using the ZBJ-ArtF1c (F) (AGATATTGGAAC*TTATATTTTATTTTTGG) and ZBJ-ArtR2c ®(*ACTAATCAATT*CCAAATCCTCC) primer set (Zeale et al. 2011). Metabarcoding sequences were also run on Illumina NextSeq 200 on a 2 x 150bp flow cell. Read processing and identification: Reads derived from PCR-free libraries were demultiplexed on Illumina basespace software. Reads were then normalized using the program bbnorm to normalize 100 depths in order to improve assembly (Bushnell 2015). Due to the short read length, we combined reads into contigs in order to form a pipeline for diet analysis we used both metaSpades and MegaHit software to assemble reads into contigs (Nurk et al. 2017, Li et al 2016). We used the default setting for both programs. Resulting contigs were then searched against the entire NCBI database using ncbi blast v2.10.1+ (see Appendix for parameters (McGinnis and Madden 2004)). Blast results were then fed into Megan6 community edition for identification (See Appendix for parameters)(Huson et al. 2016). For the sake of a conservate estimate of identification diet items we only accepted taxonomic identifications at the genera level.

Metabarcoding reads were processed using the Anacapa Toolkit with some modifications (Curd et al. 2019). First, sequences were trimmed using fastp and quality sorted using the fastx-toolkit (Chen et al. 2018, Gordon and Hannon 2010). dada2 was used for denoising, dereplicating, merging and removing any chimeric sequences Callahan et al. 2016). Taxonomy was assigned using Bowtie 2 and a Bayesian Least Common Ancestor algorithm (Langmead and Salzberg 2012).

Diet analysis

To determine which local factors might influence the diet breadth and prey item choices of *P. melanarius* we calculated the richness of diet items detected at both the family and genera level. These metrics were then regressed against % ground cover and plant height. To do so, we used generalized linear mixed effects models with a gaussian distribution via the glmmTMB package in R (Brooks et al 2017). We included farm as a random effect in all models. Models were run using the Bobyqa optimizer. We constructed a model set consisting of all single variable and additive models with the '% ground cover' and 'plant height' variables as fixed effects. All 7 models in our set included farm as a random effect and 'Method' as a fixed effect (Appendix Table 1). To assess the impact of explanatory variables on the specifics of P. melanarius diet, we used the mantel function from the vegan package (Dixon 2003). Explanatory variables evaluated were individual beetle length, plant height, and % ground cover. The Euclidean distance between each individual beetle was calculated for each variable and a Spearman correlation performed.

Results

Contig generation comparison

PCR-free library preparation yielded 519 million reads, while metabarcoding library preparation yielded 953 million reads. Contig generation with metaSPAdes and MegaHit was variable with MegaHit producing more contigs (-1.08460 ± 0.08446, $p < 0.0001$) (Figure 3.2A) as well as longer total assemblies (-0.9213 ±0.0447, $p <0.0001$.) (Figure 3.2B). However, metaSPAdes produced contigs with significantly higher N50s (0.37998 ± 0.0395 $p < 0.0001$) (Figure 3.2C). Identifications of contigs generated by MetaSPAdes and MegaHit resulted in 4,689 and 15,160 total alignments respectively. Metabarcoding based identifications yield 72,319 identifications which filtered down to 42,635 assignments after filtering for identification confidence. Table 3.1 shows the breakdown of the comparison between identifications between both Contig generation methods and metabarcoding based approach.

Diet analysis

For total family level and genera level classifications identified across all samples, shotgun metagenomics yielded more identifications. However, for Insecta specific family and genera, metabarcoding yielded more identifications (Table 3.1). Necessarily, the majority of identifications were bacterial (Figure 3.3) and even when restricted to Eukaryotic taxa, insecta identifications, while a majority, were not as dominant as they were in metabarcoding. (Figure 3.4). Metagenomic methods had lower insect family level richness when compared with metabarcoding (metaSPAdes: -32.76074 ± 1.20359 families, $p < 0.001$; MegaHit: -36.99 ± 1.20359 families, $p < 0.001$). Contigs generated with metaSPAdes also had lower richness when compared to those generated by MegaHIt (-4.233 ± 1.204, $p = 0.0013$) (Figure 3.5). . Sample size limitations prevented meaningful analysis of the impact of local factors on family level diet richness (Appendix Figure 1). Insect genera richness followed a similar pattern with metagenomic methods again having lower richness when compared to metabarcoding (metaSPAdes: -58.208 ± 1.771, $p < 0.001$; MegaHit: -53.008 ± 1.771, $p < 0.001$). Similarly, contigs generated by metaSPAdes showed lower genera level richness (-5.200 ± 1.77, $p = 0.00956$) (Figure 3.6).

The composition of general level diet items identified in the guts of *P. melanarius* across the farms was not correlated with differences of on farm ground cover ($R = 0.02$, $p = 0.37$, mantel test) or individual body size ($R = -0.0006$, $p = 0.495$) for contigs generated by metaSPAdes. Crop height approached significance but was a weak explanation of diet composition ($R = 0.0689$, $p = 0.0935$). Contigs generated by MegaHit had similar results for ground cover ($R = 0.02$, $p = 0.37$, mantel test), body size ($R = -0.006$, $p = 0.496$) and crop heigh ($R = 0.0698$, $p = 0.0976$). Identifications from metabarcoding followed a same pattern with ground cover ($R = 0.0288$, $p = 0.369$), body size ($R = -0.006$, $p = 0.503$), and crop height ($R = 0.0981$, $p = 0.0957$) having identical patterns. Condensing to the family level saw similarly non explanatory results for both contig generation methods for ground cover (metaSPAdes: $R = 0.0315$, $p = 0.36$; MegaHit: $R = 0.0425$, $p = 0.6136$, mantel test), body size (metaSPAdes: $R = -0.064$, $p = 0.702$;

MegaHit: R = -0.01308, p = 0.4999, mantel test), and crop height (metaSPAdes: R= 0.0332, p = 0.2554; MegaHit: R = 0.05297, p = 0.1508). Metabarcoding also followed a similar pattern to genera level diet items for ground cover (R = -0.03633, p = 0.6557, mantel test), body length (R = 0. 09536, p = -.1497) and crop height (R = 0.1294, p = 0.377).

Discussion

Combining shotgun metagenomics and metabarcoding identified several prey items across a broad range of taxa (Table 3.1). The majority of identifications from shotgun metagenomics were bacterial as opposed to metabarcoding which, unsurprisingly, were restricted to eukaryotes and the class Insecta specifically (99% of these insects were successfully identified; Figure 3.4). The taxa detected included several genera which are of import to brassica biological control system (Table 3.2). Metabarcoding was superior at identifying taxa closely related to *P. melanarius*. These genera (*Agonum*, *Amara*, *Anisodactylus*, *Bembidion*, *Carabus*, and *Harpalus*) have been found to be either weed seed predators or potential biological control agents in their own right (Cividanes 2021, Ward et al. 2011, Lundgren and McCravy 2011). These identifications could also have been a result of misattribution as it is unclear how well the general arthropod primer used would parse among carabid genera. However, pest taxa were not as well represented, with only *Drosophila* being detected in any of the beetles sampled. In contrast, shotgun metagenomics identified several prey genera which contain prominent agricultural pests. *Diabrotica* in particular represents a pest which hasn't been identified as a prey item of *P. melanarius*: the spotted cucumber beetle, *Diabrotica undecimpunctata* Mannerheim (Coleoptera: Chrysomelidae). While *D. undecimpunctata* is a foliar pest as an adult, the larvae are root feeders and so could have been consumed by *P. melanarius* either above- or below-ground (EFSA et al. 2020, Krysan 1986). In addition, *Leptinotarsus* was also detected via both methods and this genus includes pests such as the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Similar to *D. undecimpunctata*, *L. decemlineata* also spends part of their lifecycle in the soil where they would be vulnerable to predation by *P. melanarius* (Boiteau and Le Blanc 1992, Lipa 1985). The reason for shotgun metagenomics revealing these diet items as opposed to metabarcoding might be due to that specific region not surviving partial digestion, while the contig generation process allowed for a broader assignment length on average than metabarcoding allowed (Table 3.1). This would in theory allow for matches against other regions and allow for more databases to be utilized, as was the case here. Shotgun metagenomics also allowed for identifying plant taxa as well, though in this case each of the identifications were of weeds commonly found in and around fields in this system (Table 3.3) (Thomas 1985).

Examining how on-farm factors impacted the diet breadth and composition of *P. melanarius* met with limited success. Once again, the metabarcoding vs shotgun metagenomics had an impact on the results. Family level and genera level richness were positively associated with higher ground cover when utilizing only the results of metabarcoding. This relationship makes some sense as increasing the local non-crop vegetation would allow for more varied diet items (Prasad and Snyder 2006, Tscharntke et al. 2016, Jmhasly and Nentwig 1995). However, metagenomic identifications yielded the opposite result, with greater ground cover yielding lower richness in diet items detected. Once again there is a logical reason, as greater complexity could be limiting the prey items that *P. melanarius* can actually catch and consume McKemey et al. 2003). The discrepancy does call for more controlled studies to help mitigate the possibility of false positives. Given the incredibly high number of identifications found via metabarcoding, it is possible that metabarcoding was leading to false positives, especially since the average overlap for identification was only 151 base pairs as opposed to the metagenomic method which produced contigs that had an average overlap nearly 10 times higher (Table 3.1). In addition, no factors we measured adequately predicted the composition of *P. melanarius* diets. This was likely due to both the sheer variety of diet items as well as the limited sample size (30 beetles over 5 farms). Such low numbers precluded logistic regressions.

While shotgun metagenomics is a promising tool for more in-depth diet analysis for predators that have a broad theoretical diet, like *P. melanarius*, there are several downsides to the technique. One major issue is the need for high DNA concentration which can be difficult to acquire from the guts of insect predators collected in the field, as many may lack enough gut content volume to reach the required concentration currently needed for current pcr-free sequencing library preparation. This resulted in a small fraction of collected beetles being used for actual analysis. In addition, while we achieved satisfactory results from using both metaSPAdes and MegaHit these contig generation programs were made and optimized around microbial communities and microbial metagenomics are the test cases used to evaluate them (Duncan et al. 2020, van der Walt et al. 2017, Narzisi and Mishra 2011, Zhang et al 2011). As such, it is unsurprising that the majority of contigs which were identifiable were non-eukaryotic. Hopefully as the means to extract DNA improve, the first hurdle will be alleviated and some software has been designed with eukaryotic genomes in mind, though still with a focus on the microbial (Karin et al. 2020). Whether current metagenomic software is adequate for eukaryotic diet analysis would require laboratory studies to confirm in a similar manner to studies used to establish how long prey items remain detectable post ingestion (Eitzinger et al. 2014, Von Berg et al. 2008). Though the bacterial identifications are not necessarily correlated to diet, the fact that these data are were also collected means that microbiome studies can be conducted in tandem with diet analysis without the need for multiple DNA extractions or sequencing runs, as well as diet identifications via obligate microbial symbiotes (Paula et al. 2016). Overall, the pipeline developed here could be a key stepping stone to increased usage of shotgun metagenomics as an alternative to metabarcoding. This will in turn allow for far more in-depth understanding of the role of generalist predators in actual field conditions.

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Figures

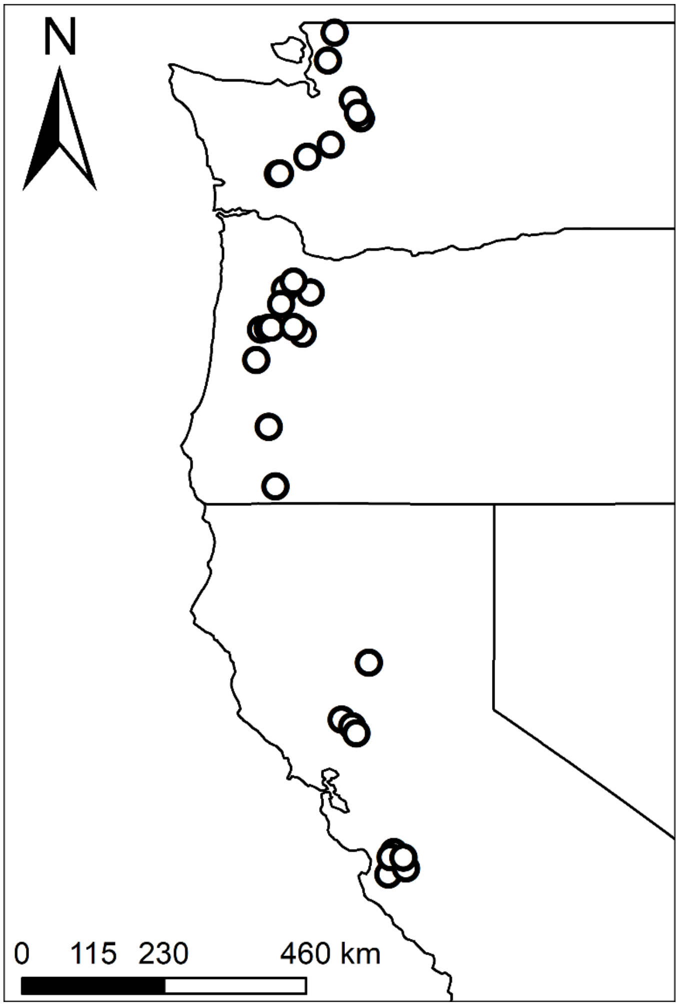

Figure 3.1. Map of farms where collections were conducted

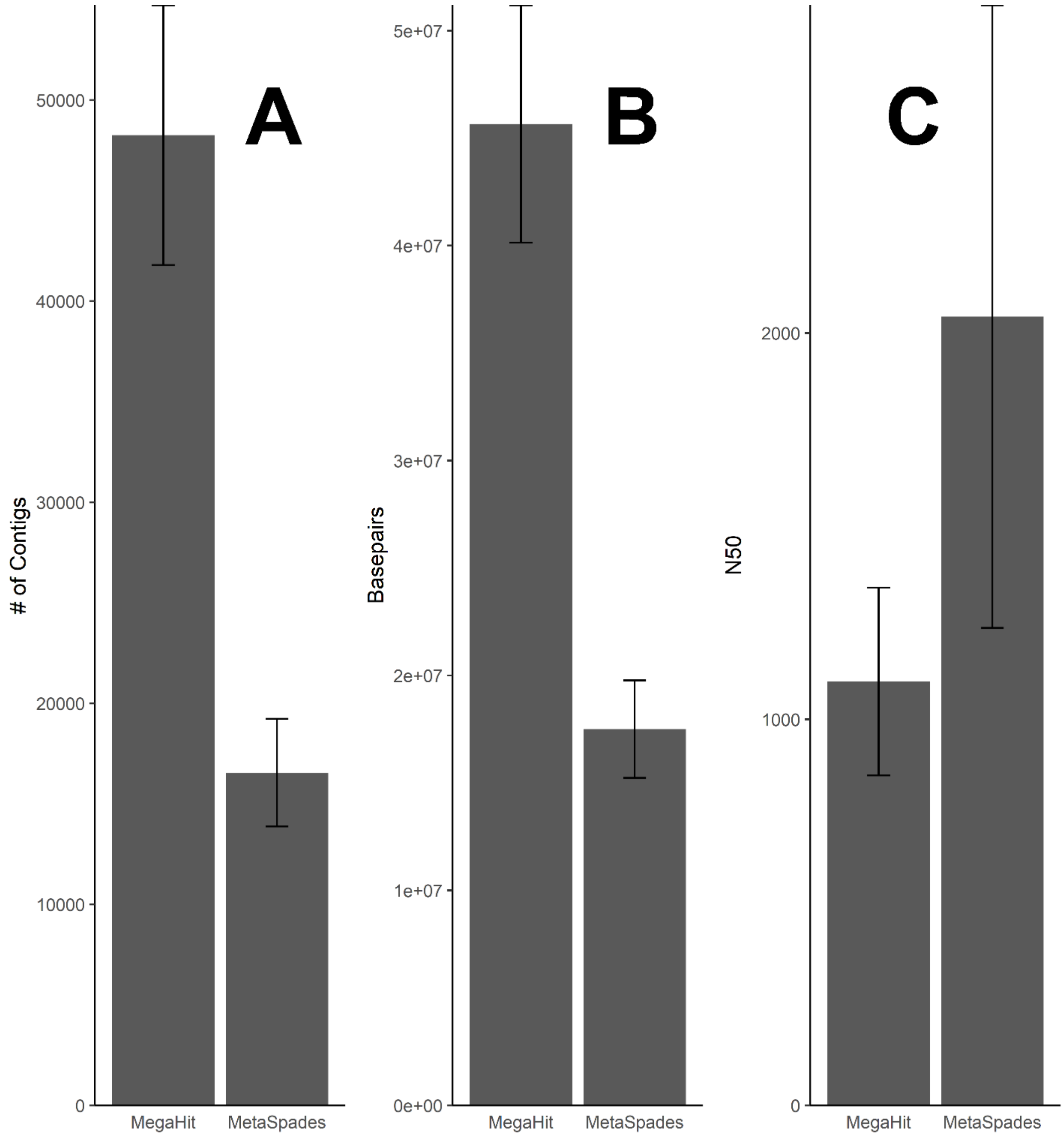

Figure 3.2. Comparison between contig generation methods. (A) Number of contigs generated by MegaHit and metaSPAdes respectively. (B) Average length of contigs generated by MegaHit and metaSPAdes respectively. (C) N50 of contigs generated with MegaHit and metaSPAdes.

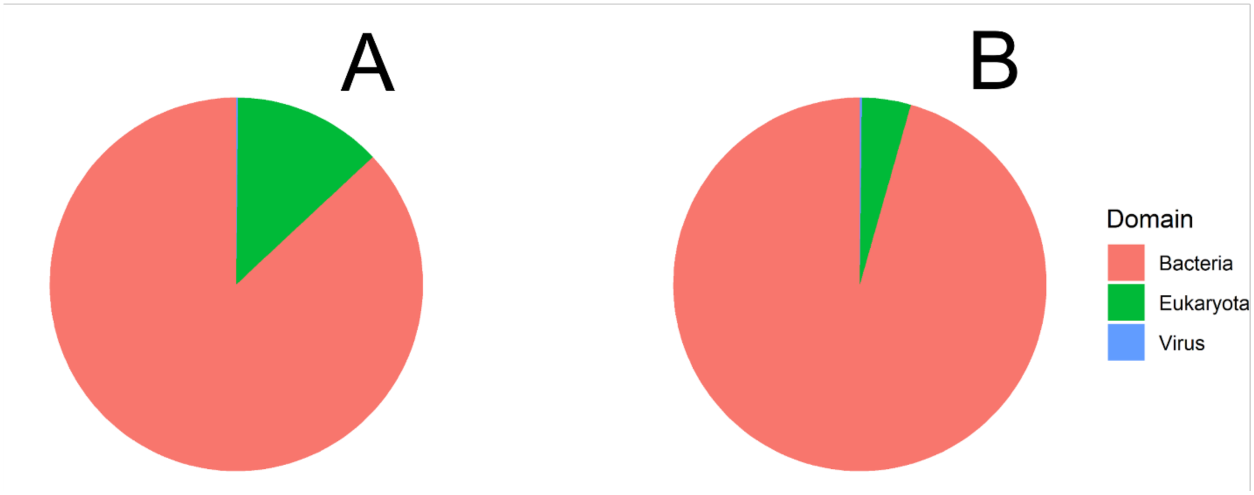

Figure 3.3. Domain level identifications for MegaHIt (A) and metaSPAdes (B).

Figure 3.4. Percentage of Identifications assigned to class for MegaHit (A), metaSPAdes (B) and Metabarcoding (C).

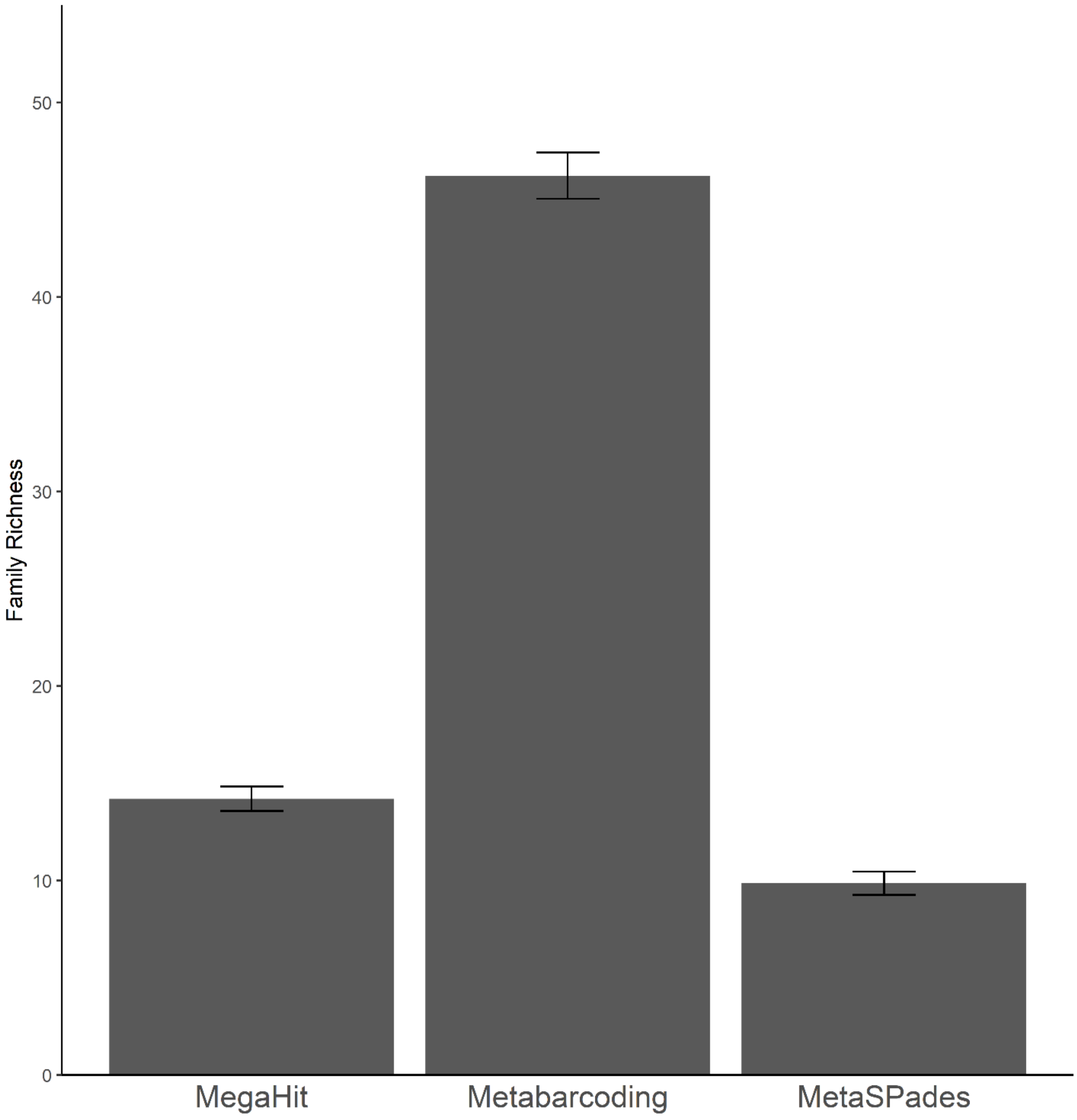

Figure 3.5. Family Richness determined from each method for generating reads or contigs

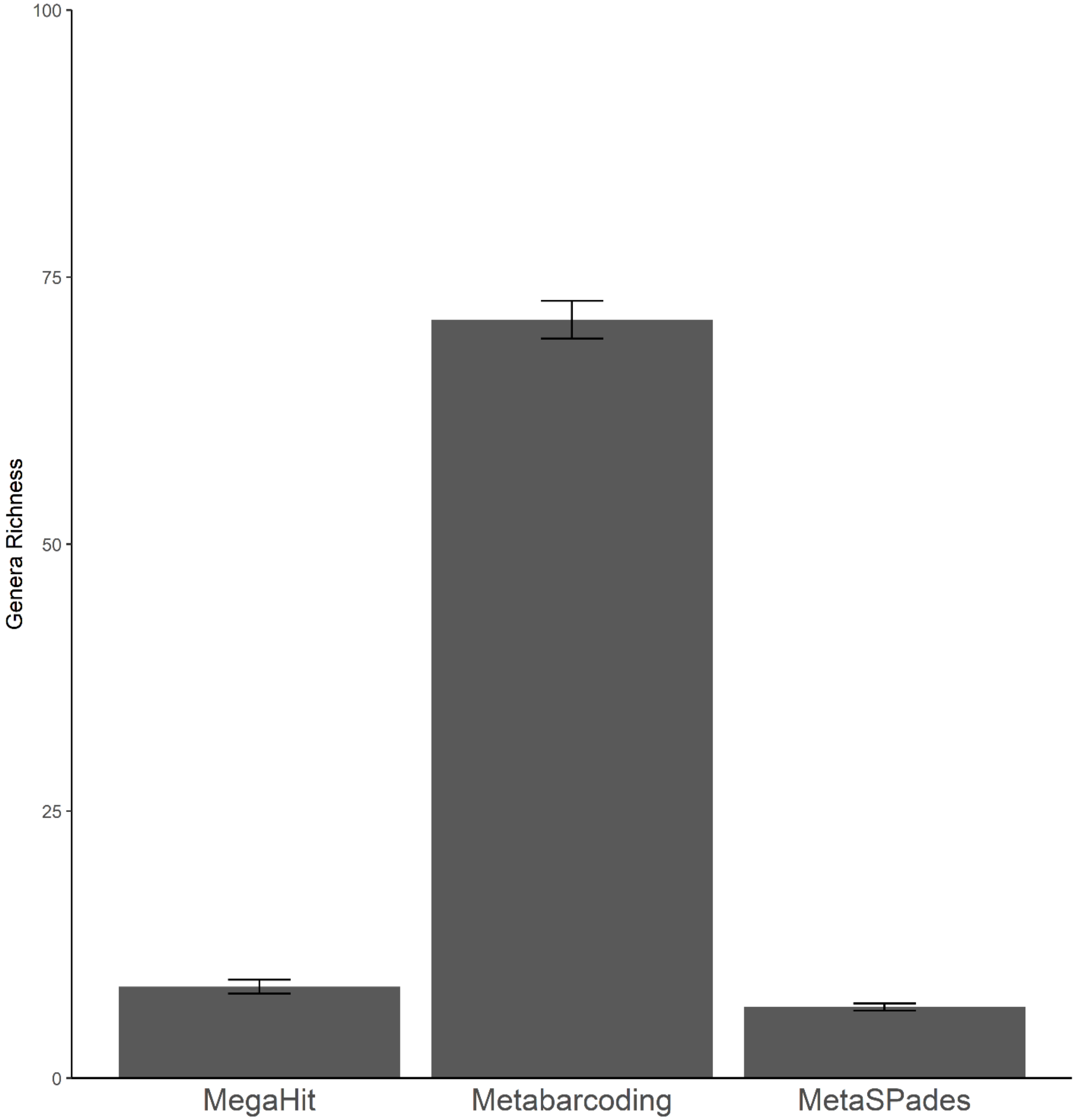

Figure 3.6. Genera richness by each method of generating reads or contigs

Tables

Table 3.1. Summary statistics comparing different Shotgun Metagenomic taxonomic identification (From contigs generated with either MetaSPAdes or MegaHit) and Amplicon based techniques.

Method	Eukaryote Alignment Length	Bacteria Alignment Length	# Family IDs	# Genera IDs	Insecta Families	Insecta Genera
MetaSPAdes	1197.264	5830.557	32	63	16	30
MegaHit	1078.08	4877.822	31	580	18	34
Metabarcoding	151	NA	173	495	145	471

Table 3.2. Number of P. melanarius with identifications of important taxa in brassica systems (percentage of P. melanarius with a detection)

Order	*Family*	*Genera*	*Agricultural Role*	*Metabarcoding*	*MegaHit*	*metaSPAdes*
Coleoptera						
	Carabidae					
		Agonum	Predator	24 (80%)		
		Amara	Seed predator	29 (96.666%)		
		Anisodactylus	Predator	30 (100%)		
		Bembidion	Predator	27 (90%)		
		Blackburnia	Predator		2 (6.666%)	
		Cycnotrachelus	Predator	28 (93.33%)		
		Dromius	Predator	26 (86.666%)		
		Harpalus	Seed predator	20 (66.666%)	3 (10%)	2 (6.666%)
		Platynus	Predator	30 (100%)		
		Synuchus	Predator	30 (100%)		
	Chrysomelidae					
		*Diabrotica**	Pest		8 (20%)	2 (6.666%)
		*Leptinotarsa**	Pest		5 (16.67%)	7 (23.333%)
	Staphylinidae					
		Aleochara	Predator	22 (73.333%)		
Diptera						
	Tephritidae					
		Bactrocera	Pest		2 (6.666%)	2 (6.666%)
Hymenoptera						
	Vespidae					
		Anicsstrocerus	Predator	1 (3.333%)		
Lepidoptera	Noctuidae					
		Spodoptera	Pest		1 (3.333%)	26 (86.666%)
	Sphingidae					
		Manduca	Pest		1 (3.333%)	1 (3.333%)

Table 3.3. Incidence of plant genera detected in P. melanarius from contigs generated using metaSPAdes and MegaHit software

Method	Chenopodium	Erythranthe	Gossypium	Lasthenia
metaSPAdes	2	0	0	3
MegaHit	3	1	1	1

Chapter 4

Weedy Brassica Fields Encourage More Diverse and Abundant Predatory Insects[3]

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[3] Taylor, J.M., Smith O.M., Snyder, W.E. Prepared for the journal Environmental Entomology

Abstract

Increasing plant diversity within agricultural fields might enhance food and shelter for natural enemies, while also making it more difficult for herbivores to locate their host plants. For example, it has been suggested that natural enemies might be more abundant, and pests less abundant, in fields where weed management is somewhat relaxed. We searched for any relationship between weediness and insect communities in 50 commercial organic *Brassica* fields across the western U.S. states of California, Oregon and Washington, over two growing seasons. Fields differed not only in weed cover, but also in the structure of surrounding landscapes and whether livestock were raised alongside vegetables. We found that greater weed cover generally correlated with greater species richness and abundance of predatory insects in crop foliage, and abundance of predatory carabid beetles on the ground. Contrary to expectations, however, we did not find any change in abundance of aphid, beetle or caterpillar *Brassica* herbivores, such that any enhancement of natural enemies did not lead to stronger net pest suppression. Pests were generally more abundant on larger *Brassica* plants, although other local and landscape factors exerted relatively little obvious impact on herbivorous or predatory insects. Overall, while weedier fields appeared to be more successful at attracting and/or retaining predators, more predators did not correlate with fewer pests. This reinforces the broader challenge in developing real-world conservation strategies that both benefit natural enemies and meaningfully improve biocontrol.

Key words: predator conservation, biological control, generalist predator, herbivore

Introduction

Natural enemies often struggle to survive in agricultural fields (Rusch et al. 2016). This is in part because monocultures of a single crop species rarely provide the diversity of food, shelter, and other resources that natural enemies require. One solution is to diversify on-farm plantings. For example, planting wildflowers along the edge of cropping fields can provide pollen and nectar for polyphagous natural enemies, attract alternative non-pest prey arthropods, and provide refuge from tillage and other disturbances (Blaauw and Isaacs 2012, Ditner et al. 2013, Šálek et al. 2018). While this often is successful in increasing natural enemy abundance and/or diversity in the refuge, it is less common to see reduced pest abundance in the adjacent crop (Symondson et al. 2002, Seidl et al. 2020). Failure of this approach to conservation biological control often results from natural enemies refusing to leave the relatively benign environment of a refuge, to move back into the crop to feed on pests there (Collins et al. 2002). Also, pests sometimes make use of the same resources as natural enemies, swamping any net benefit from greater predator abundance (Tscharntke et al. 2016).

A more effective technique may be to provide additional resources within the cropping field itself, so that natural enemy movement between field and refuge is not necessary. For example, enhancing soil fertility with animal or plant manures, rather than synthetic-chemical fertilizers, can provide food for detritus-feeding alternative prey for natural enemies that strengthens biological control (Muneret et al. 2018, Aldebron et al. 2020). Intercropping several different crop species can similarly diversify the resources available to natural enemies. Perhaps the simplest approach to in-field crop diversification is to relax weed suppression (Landis et al. 2005, Blubaugh et al. 2016). This can carry an obvious cost if weeds heavily compete with crops to reduce yield, although this harm may be at least partially counteracted by enhanced natural abundance and predation on pests (Landis et al. 2005). As an additional benefit for natural pest control, crops nestled among weeds may be more difficult for pests to locate using chemical or visual cues (Smith and McSorley 2000, Finch and Collier 2012). There is the risk, however, that the greater diversity of prey in weedy fields will only draw predator attacks away from pests, or that pests will also benefit from the food and shelter that weeds provide (Dabney et al. 1996, Maas et al. 1998). So, weedier fields might experience greater crop competition without any net benefit to biological control (Blubaugh et al. 2021).

Here, we consider the impacts of in-field weediness, and also several other aspects of farm and landscape diversity, on predatory and pest insects in commercial *Brassica* fields. Our study sites were 29 fields managed by cooperating growers in the western US states of Washington, Oregon and California. All farms used organic methods, but 14 produced livestock (usually chickens) alongside vegetables rather than growing crops alone. Integrated livestock farms generally house a greater diversity of on-farm habitats (Kremen et al. 2012), and farms of both types were embedded in landscapes that ranged from primarily agricultural to primarily natural(Figure 1). So, in addition to weediness, farms also experienced differences in habitat diversity at the local and landscape scales known to impact predatory and herbivorous insects (Hooks and Johnson 2003, Bischoff et al. 2016). We hypothesized that greater weed cover enhance abundance and biodiversity of predatory insects, while discouraging herbivorous pests. However, we also acknowledged that any in-field effects of weediness might be overwhelmed by the effects of greater habitat diversity at the farm or landscape scales.

Materials and Methods

Study System

West coast brassica systems are home to a suite of common pests, including *Pieris rapae Linnaeus* (Lepidoptera: Pieridae)*, Plutella xyllostella* Linnaeus (Lepidoptera: Plutellidae), *Tricoplusia ni* Hübner (Lepidoptera: Noctuidae), *Myzus persiae* Sulzer (Hemiptera: Aphididae), B*revicoryne brassica* Linnaeus (Hemiptera: Aphididae), and *Phyllotreta cruciferae* Goeze (Coleoptera: Chrysomelidae) (Lamb 1989, Brown et al. 1999). The system is also host to several predators including the ground beetle *Pterostichus melanarius* Linnaeus (Coleoptera: Carabidae), green lacewings (*Chrysoperla sp.* Steinmann (Neuroptera: Chrysopidae) as well as the predator hemipteran *Nabis* sp. Latreille (Hemiptera: Nabidae) (Ma et al. 2005). Previous work on a smaller subset of farms in this system has suggested that increased ground cover benefits ground predators and that this might lead to better control of *P. crucifera* (Blubaugh et al. 2021) However, the foliar predator community, as well as the broader pest community was not considered there. In addition, we consider both vegetable farms and those which integrated livestock. Integrated farms were those that raised some form of livestock (the majority of which were chickens) as described in previous work in the system (Smith et al. 2020, 2021). We surveyed arthropods at 29 farms across 3 states (Washington, Oregon, and California). Farms were split between organic vegetables integrated with livestock production (N=14) and organic vegetable production in the absence of livestock (N=15).

Landscape Context

To characterize landscape context, we calculated the percent natural/semi-natural (hereafter, “seminatural”) cover based on the 2016 National Land Cover Database (Homer et al. 2012) using a 1000 m radius buffer centered at the farm center using Program R and

FRAGSTATS 4.1 Natural cover included forest (deciduous, evergreen, and mixed), scrubland (dwarf scrub and shrub/scrub), herbaceous (grassland/herbaceous, sedge/herbaceous, lichens, and moss), and wetland categories (woody and emergent herbaceous wetlands). Categories not included in natural cover were water, ice/snow, developed, barren, pasture/hay, and cultivated crop classes. We used a 1000 m buffer to encompass the daily activity centers for arthropods because it is commonly used for arthropod assessments in agricultural systems (Lichtenberg et al. 2017, Billerman et al. 2020, Tamburini et al. 2020)

Cover Quantification

Percent cover was quantified on each farm by taking 10 equally spaced picture transects with a 1 x 1 meter Daubenmire frame. Each picture transect was broken down into a 100 square grid and the percentage of non-bare ground was calculated. The % cover from each transect was then averaged for each farm.

Insect Surveys

In the brassica portion of each farm we conducted visual surveys of brassica plants available. Kale and broccoli were surveyed separately. Surveys consisted of comprehensive examination of each leaf with care taken for minimal disturbance of plants. Insects were visually identified to the lowest taxonomic rank possible. For the majority taxa this was the family level but common brassica pests *(B. brevicoryne, P. rapae*, *P. xyllostella, T. ni* and *M. persicae*) were identified to the species level. In addition, 10 other plants were haphazardly selected for insect sampling via Dietrich Vacuum (D-vac) Each plant was sampled for 10 seconds each. Filled collection bags were frozen via dry ice and immediately placed in 70% ethanol for later identification. In addition, we measured ground beetle activity density via pitfall trapping. 5 pitfalls were placed every 2 meters or equally spaced in fields less than 10 meters across. Pitfall transects were also placed as near to the center line of the field as was possible in order to limit edge effects. Pitfalls were set during the evening (from 17:00-19:00) and collected the following morning. Pitfalls were "wet" and filled approx. halfway with water denatured with organic dish soap. Captured invertebrates were stored in 70% ethanol and later identified to the genera level for carabid beetles. We used the vegan package in R to calculate richness as well as Shannon's diversity index "H" at the family level in order to account for the limitations at identifying some taxa (Oksanen et al. 2009). Evenness was calculated using the formula Shannon's H/log(richness) and was calculated for all insects surveyed.

Statistical analysis

To examine the importance of livestock presence, survey period, and percent seminatural cover in the landscape, percent natural cover in the landscape, plant height and average ground cover on insect community we compared all nested models from our global model. We constructed a complete model set consisting of an intercept-only null model as well as all single variable and additive models with the 'Livestock', '%local natural habitat', '% landscape seminatural habitat', 'plant height', 'average ground cover', and 'survey round' variables as fixed effects (Appendix Table 2). All 22 models in our set included plot nested within farm as a random effect. Dependent variables examined were total insect abundance, total insect family richness, total insect evenness, foliar predator family richness, coccinellidae abundance, nabidae abundance, carabidae activity density, aphid density, P. *crucifirae* abundance, P. *rapae* abundance, and P. *xyllostella* abundance. In addition, Foliar predator family richness was tested as an explanatory variable against pest abundances. In addition, in order to prevent methodological differences from influencing results, with the exception of carabidae and the aphid/plant metric, all insect measurements are referring to individuals collected via D-vacing.

We compared models based on AICc in the bbmle package in R and identified the top-competing models as those within ΔAICc < 2.0 of the most well-supported model(Burnham and Anderson 2004, Bolker and Team 2010). We then estimated covariate effects by model averaging among the best-supported models (within 2 ΔAICc of the best-supported model) using the model.avg function in the MuMIn package in R (Burnham and Anderson 2004, Bolker and Team 2010). We considered covariates as strong predictors of the response variables if they appeared in the top models (ΔAICc < 2.0) and their model-averaged 95% confidence intervals did not overlap zero.

Results

Community metrics

Evenness was not predicted by any tested variables with the null model being the most supported (Figure 4.2 A,B; Appendix Table 3). Total insect richness was higher with increased ground cover ($\beta = 0.0086 \pm 0.0032$) but was not significantly influenced by plant height (Figure 2C,D; SI Table 2). Total insect abundance was positively associated with increasing ground cover ($\beta = 0.0126 \pm 0.0065$) (Figure 2E,F; Appendix Table 3). Foliar predator richness was positively correlated with increasing ground cover ($\beta = 0.013807 \pm 0.005138$) but not plant height or survey period (Figure 2G,H; Appendix Table 3).

Natural enemies

Coccinellidae abundance was positively associated with increasing ground cover though the model average only approached significance ($P = 0.0636$, $\beta = 0.0198 \pm 0.0105$) (Figure 3A,B; Appendix Table 3). Carabid activity density was positively associated with increased ground cover ($\beta = 0.0201 \pm 0.0098$) and had no significant association with plant height (Figure 3C,D; Appendix Table 3). Nabidae abundance was negatively associated with % seminatural habitat (β = -2.385 ± 1.266) and was not significantly associated with the presence of livestock, ground cover, plant height, or survey period (Figure 3E,F; Appendix Table 3).

Pest Taxa

Aphids/plant was positively associated with the later survey period (β = 1.175243 ± 0.540207) and Plant height (β = 0.005232 ± 0.002015) and a negative correlation with livestock presence was approaching significance (β = 0.013807 ± 0.005138) (Figure 4A,B; Appendix Table 3). P. *crucifierae* abundance was positively associated with plant height (β = 0.015076 ± 0.004656) and was not significantly impacted by survey period, ground cover, or % local natural habitat (Figure 4C,D; Appendix Table 3). P. *rapae* abundance had a positive association with ground cover (β = 0.02266 ± 0.01166) but the relationship was only approaching significance (P =0.0570) (% Natural and survey period did not have any significant effects (Figure 4E,F; Appendix Table 3). P. *xyllostella* abundance was positively associated with plant height (β = 0.013807 ± 0.005138) as well as the presence of livestock (β = 0.999615 ± 0.59369) though this only approached significance (P = 0.09936). Survey Period, ground cover, and % natural habitat had no significant effect (Figure 4G,H; Appendix Table 3). Foliar predator richness was not shown to be a correlated to the abundance of any pest (Appendix Table 4)

Discussion

Though previous studies have shown evenness to be beneficial (Crowder et al. 2010, 2012), insect community evenness appeared uncorrelated with local or landscape level factors in our study. Based on previous studies it seems like the presence of particularly impactful predators, such as *P. melanarius*, has a stronger impact on evenness than the habitat or landscape (Blubaugh et al. 2021). Total abundance, total richness, and foliar predator richness all were positively associated with increased ground cover. This is in line with other studies and is likely due to the increase of resources available with a more varied habitat (Letourneau et al. 2011a). Obviously, this is positive evidence that habitat alterations can increase suitability of crop fields as habitat for native predators. However, whether this would translate to an increase in biological control was beyond this study. Previous work has shown mixed results in this regard and whether yields would be increased is likely system specific (Lichtenberg et al. 2017, Muneret et al. 2018, Taylor et al. 2022).

When broken down by taxa, we found that natural enemies were positively affected by local factors. Both coccinelidae and carabidae, both of which have been highlighted as biological control agents in brassica systems as well as agriculture as a whole (Obrycki and Kring 1998, Obrycki et al. 2009, Lundgren and McCravy 2011), benefited from greater weed cover. The positive effect of ground cover on these two taxa is supported by other work in similar systems (Letourneau et al. 2011b, Lichtenberg et al. 2017). However, we saw no benefit to *Nabis* which has been shown to instead benefit from weedy vegetation in other agroecosystems (Elliott et al. 1999, Kheirodin et al. 2022). The disagreement here could be the result of a relatively mild effect coupled with a low *Nabis* abundance in our study. This could have been partially driven by the surrounding landscape not being conducive to supporting *Nabis* populations. Similar explanations could be used to explain the negative relationship with surrounding neighboring habitat and *Nabis* abundance (González et al. 2022).

Pest taxa showed little association with the surrounding landscape as well. This is somewhat at odds with existing literature in which landscape composition has been shown to impact cruciferous pests, though the responsiveness varied by taxa (Perez-Alvarez et al. 2018). The broad categorization of the surrounding landscape might have played a role as well as the specific composition has been shown to be important to altering the insect pest community

(Bischoff et al. 2016). It is possible that a finer distinction in landscape classification might have revealed pattern similar to that seen in previous studies (Östman et al. 2001, Martínez-Núñez et al. 2020). Pest abundance and/or density were dictated mostly by crop maturity as opposed to local or landscape metrics. This might be due to the specific taxa involved and the brassica system. The two most abundant pest groups detected, flea beetles and aphids, are known to have high densities in a suitable environment (Hughes and Gilbert 1968, Elmstrom et al. 1988). However, in brassicae plants vulnerability to such pests is highest when crops are younger and therefore smaller so this correlation is unlikely to equate to poorer crop outcomes (Cook et al. 2006, Gu et al. 2007). While the increased ground cover appeared to benefit P. *rapae* abundance, the low magnitude over the course of our study means that it was unlikely they produced a significant amount of pest pressure (Mailloux and Belloncik 1995). The positive association here is opposed to previous work, which indicates that the positive effect we found here was possibly due low numbers overall (Dempster 1969). Despite predator richness being shown to reduce pest abundance in other studies (e.g., Crowder and Jabbour 2014, Krey et al. 2021), we saw no such effect in our study.

Despite the benefits to the predator abundances seen with increased ground cover, we did notobserve a corresponding decline in pest abundance. This lack of follow through effect has been seen in other studies where increased predator abundance or richness did not lead to better natural pest control (Birkhofer et al. 2008). This has been attributed to several factors such as intraguild predation and non-target feeding, both of which could have been the reason for a lack of controlling effect here (Wyckhuys et al. 2013, Tscharntke et al. 2016). This might be particularly true for broadly polyphagous biocontrol agents such as generalist predators (Taylor and Snyder 2021).

Overall, our findings here reflect the mixed effects that local and landscape factors have been shown to have on insect communities (Clough et al. 2007, Martínez-Núñez et al. 2020). Local habitat changes benefitted some natural enemies and benefitted predator richness but failed to correlate to lower pest abundances. An important factor was that pest species did not respond in tandem. Therefore, factors that improved natural enemy abundance did so without also improving pest abundance. This underscores the premise that conservation biological control is often successful in enhancing predator abundance without also improving pest suppression. This study did not investigate whether any of the alterations to the insect community would have impacted crop yield, but given the study system this likely would have been negligible (Taylor et al. 2022). However, the specific management choices will depend on the crop system as well as the surrounding landscape.

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Figures

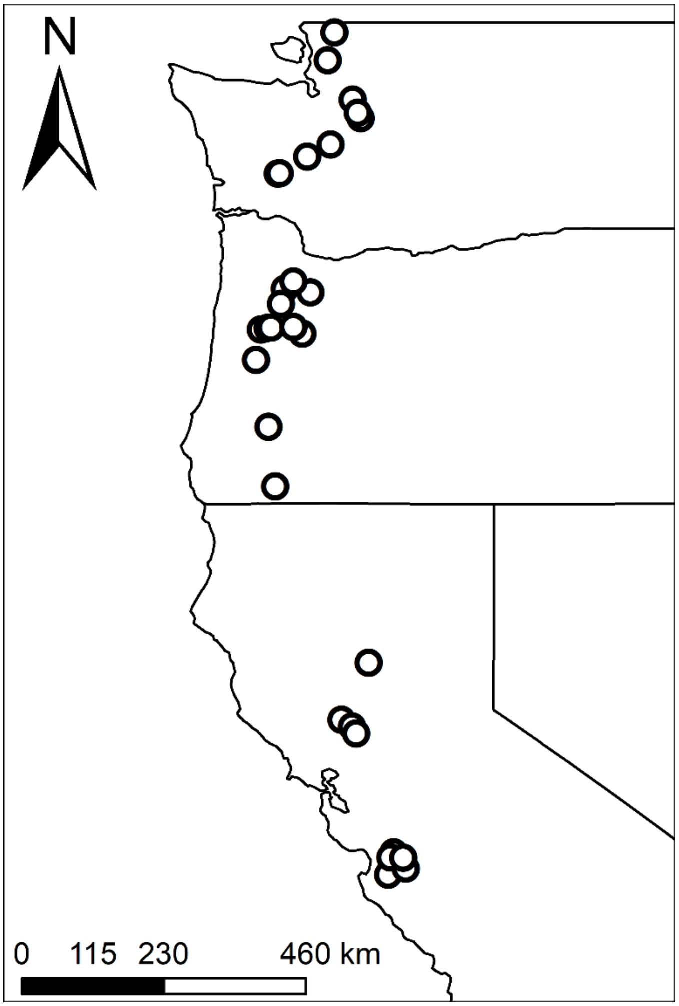

Figure 4.1. Map of the 29 farms sampled over the course of 2017-2018

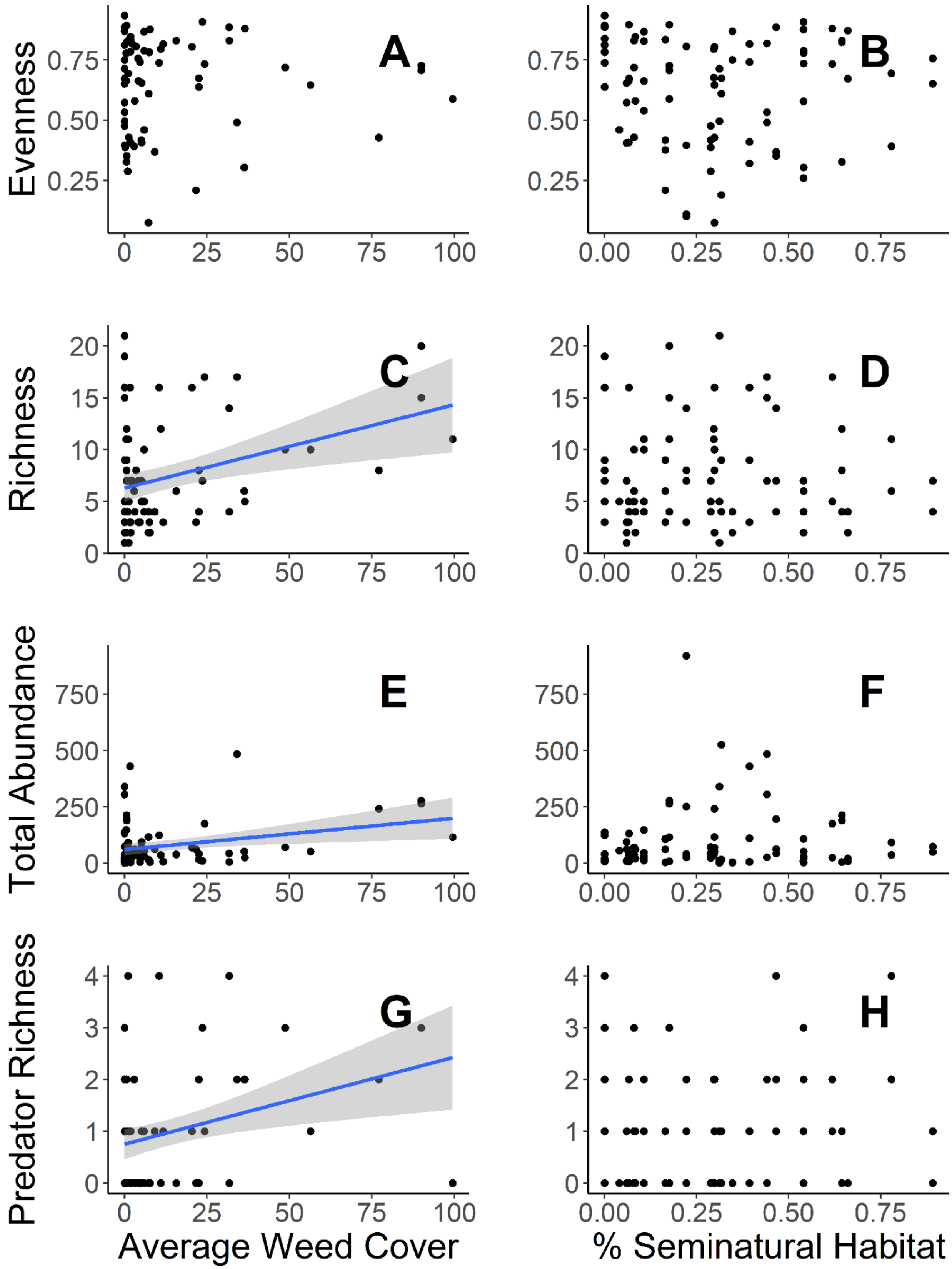Evenness
0.75
0.50
0.25
A
B
0
25
50
75
100
0.00
0.25
0.50
0.75
Richness
20
15
10
5
0
C
D
Total Abundance
750
500
250
0
E
F
Predator Richness
4
3
2
1
0
G
H
Average Weed Cover
% Seminatural Habitat

Figure 4.2. Scatterplots indicating relationships between community metrics on insect family evenness (A,B), richness (C,D), total abundance (E,F) and predator richness (G,H). All metrics are based off of D-vac samples taken from 10 plants in relation to average ground cover and % seminatural habitat. Grey shading indicates a 95% confidence interval around the regression line

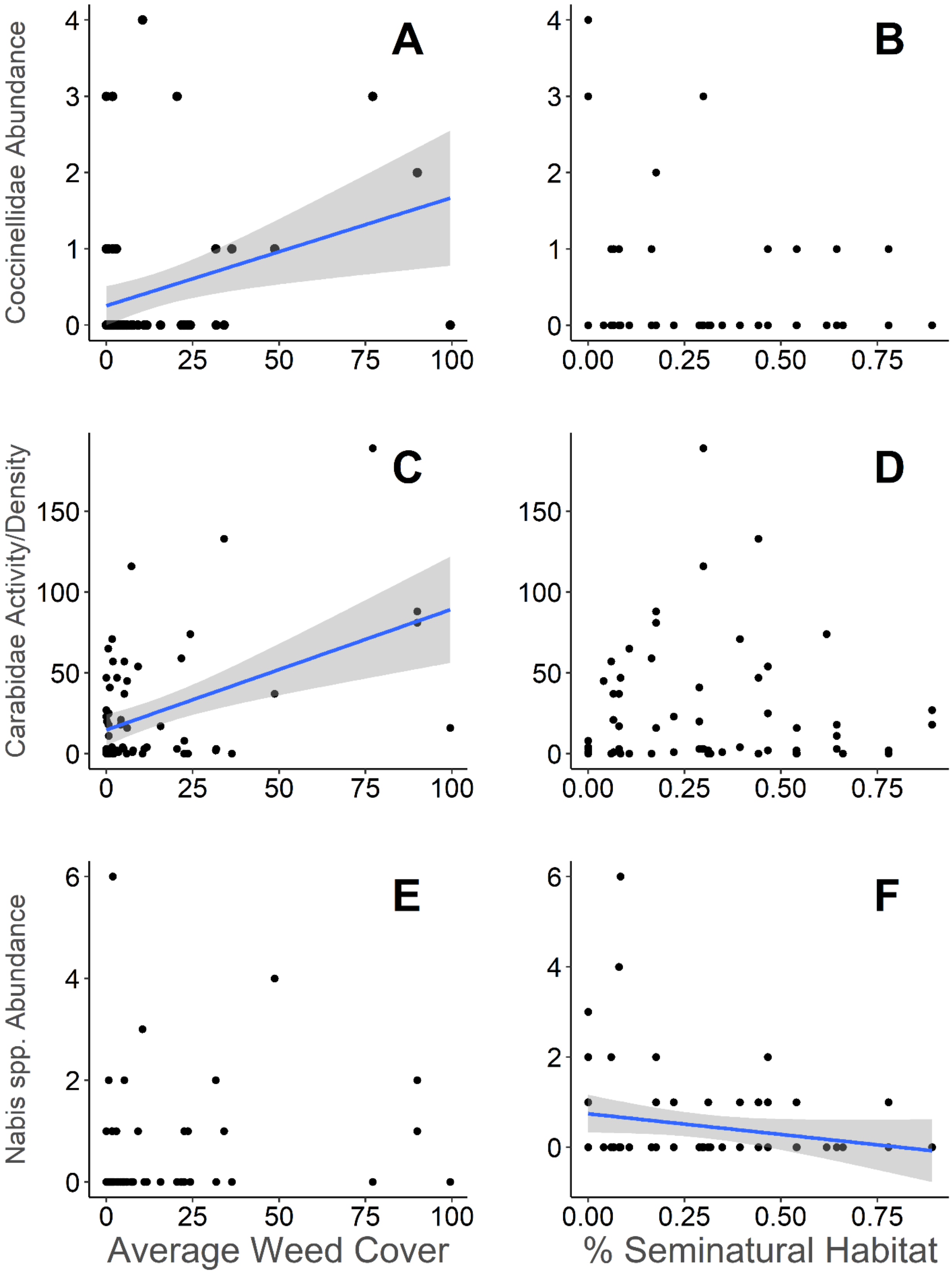
A
B
C
D
E
F
Coccinellidae Abundance
Carabidae Activity/Density
Nabis spp. Abundance
Average Weed Cover
% Seminatural Habitat
0
1
2
3
4
50
100
150
6
0
25
50
75
100
0.00
0.25
0.50
0.75

Figure 4.3 Scatterplots indicating relationships Coccinellidae (A,B), Carabidae activity-density (C,D), and *Nabis* abundance (E,F). All metrics are based off of D-vac samples taken from 10 plants in relation to average ground cover and average plant height. Grey shading indicates a 95% confidence interval around the regression line

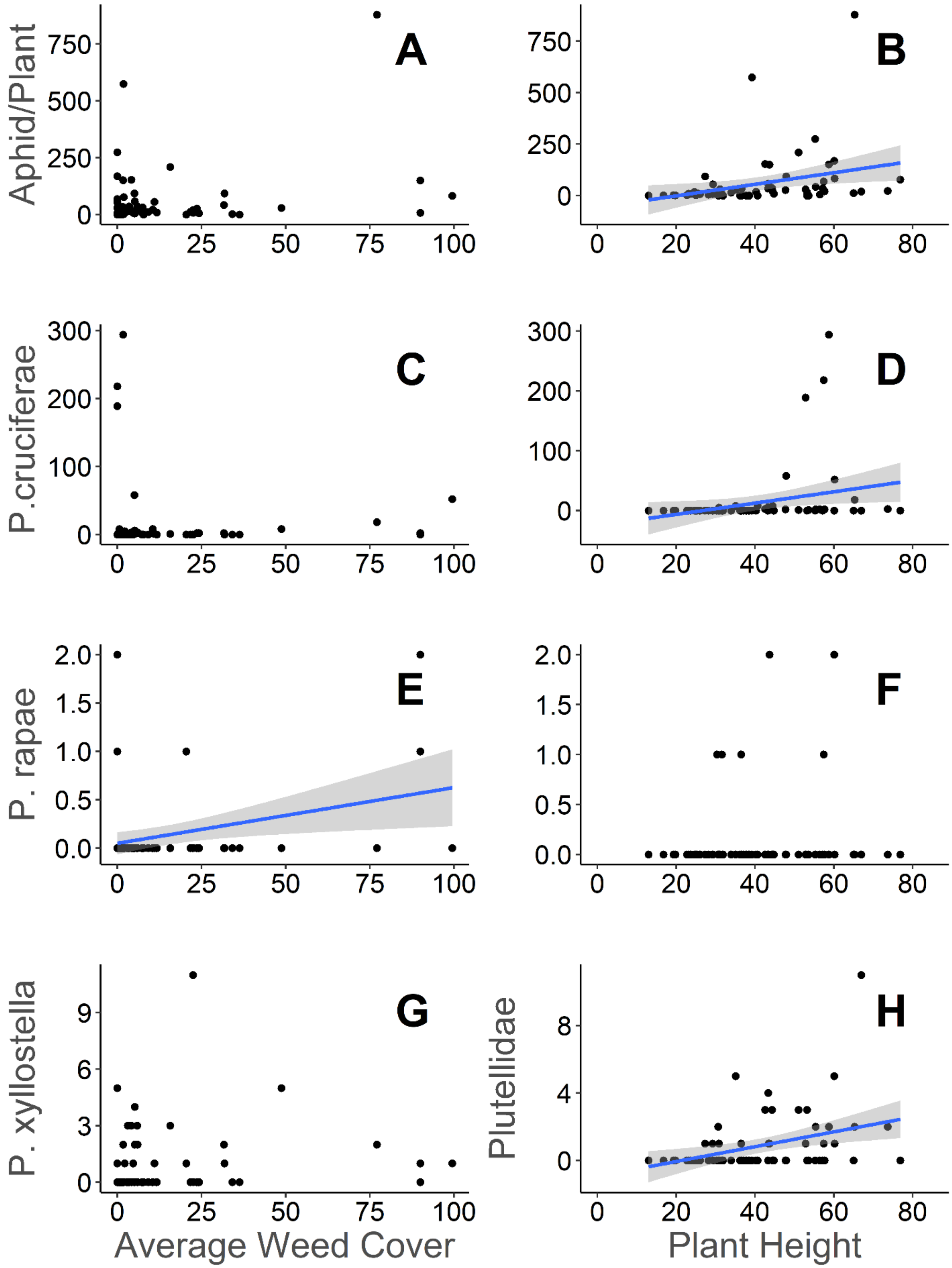
A
B
C
D
E
F
G
H
Aphid/Plant
P.cruciferae
P. rapae
P. xyllostella
Plutellidae
Average Weed Cover
Plant Height
750
500
250
0
300
200
100
2.0
1.5
1.0
0.5
0.0
9
6
3
8
4
25
50
75
100
20
40
60
80

Figure 4.4. Scatterplots indicating relationships between aphids per plant (A,B), P. *cruciferae* abundance (C,D), P. *rapae* abundance (E,F), and *P. xyllostella* abundance (G,H). All metrics are based off of D-vac samples taken from 10 plants in relation to average ground cover and average plant height. Grey shading indicates a 95% confidence interval around the regression

Chapter 5

Bird predation and landscape context shape arthropod communities on broccoli[4]

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[4] Taylor, J.M., Smith, O.M., Edworthy, M., Kennedy, C.M., Latimer, C.E.,Owen, J.P., Wilson-Rankin E.E., and Snyder 2022 Ornithological Applications 124(2): duac05 Reprinted here with permission of the publisher

ABSTRACT

Birds increase crop yields via consumption of pests in some contexts but disrupt pest control via intraguild predation in others. Landscape complexity acts as an inconsistent mediator, sometimes increasing, decreasing, or not impacting pest control. Here, we examined how landscape context and seasonal variation mediate the impact of birds on arthropod pests and natural enemies, leaf damage, and yields of broccoli (*Brassica oleracea*) on highly diversified farms that spanned the USA West Coast. Our study had two complementary components: a bird exclusion experiment and molecular diet analysis on 357 fecal samples collected from the most commonly captured bird species that foraged in *Brassica* fields-American Goldfinch (*Spinus tristis*), American Robin (*Turdus migratorius),* Savannah Sparrow (*Passerculus sandwichensis)*, Song Sparrow (*Melospiza melodia)* and White-crowned Sparrow (*Zonotrichia leucophrys*). Bird access yielded higher, rather than lower, numbers of pest aphids and increased their parasitism, while no other arthropods examined were consistently impacted. Independent of bird presence, percent natural cover in the landscape sometimes increased and sometimes decreased densities of arthropods in the mid-growth period, with diminishing impacts in the late-growth period. Herbivore feeding damage to broccoli leaves decreased with increasing amounts of natural land cover and in the late-growth period. Molecular diet analysis revealed that Brassica pests and predatory arthropods were relatively uncommon prey for birds. Landscape context did not alter the prey items found in bird diets. Altogether, our bird-exclusion experiment and molecular diet analysis suggested that birds have relatively modest impacts on the arthropods associated with broccoli plantings. More broadly, the limited support in our study for net natural pest control services suggests that financial incentives may be required to encourage the adoption of bird-friendly farming practices in certain cropping systems.

Keywords: agroecosystems, biological control, birds, ecosystem services, intraguild predation, landscape ecology, molecular scatology

LAY SUMMARY

- Birds may contribute pest control service through consumption of arthropod pests or disrupt pest control through predation on arthropod natural enemies. The net effect of birds on crop pests and subsequent yields may be mediated by landscape context.
- To better understand the net effects of birds on broccoli, we combined a bird exclusion experiment with DNA-based diet analysis of five key bird species.
- Both birds and landscape context impacted arthropods on broccoli plants. However, we observed no net effect of birds on broccoli yield regardless of landscape context.
- DNA-based diet analysis revealed that while landscape did not alter the likelihood of bird species consuming pests or arthropod natural enemies, some bird species were more likely than others to consume arthropod natural enemies.
- The limited impacts of birds on broccoli leaf damage and yields suggested that pest control services by birds may be an insufficient motivator to employ bird-friendly farm practices.

INTRODUCTION

Wild birds provide a complex mix of ecosystem services and disservices to agriculture (Pejchar et al. 2018). To the benefit of crop production, birds feed on herbivorous insects and so can help control pests (Bock et al 1992; Karp et al. 2013; Boesing et al. 2017). However, birds may also disrupt pest control through intraguild predation (Martin et al. 2013; Olimpi et al. 2020). The net impact of birds on pest control, crop damage, and subsequent yields may be mediated by landscape context (Martin et al. 2013; Boesing et al. 2017) due to changes in community composition that alter biotic interactions (Tscharntke et al. 2012; Martin et al. 2013; Karp et al. 2016). This complexity may underlie the great variation between studies on the impacts of landscape composition on pest and enemy abundances, predation rates, crop damage, and yields (Chaplin-Kramer et al. 2011; Tscharntke et al. 2016; Karp et al. 2018). For example, greater amounts of non-crop cover in the landscape showed no consistent impacts on pest management and subsequent yields in a recent global meta-analysis, although comparing similar crop and landscape features improved predictions (Karp et al. 2018). This highlights the need to examine context-dependent impacts of landscape elements on bird-mediated pest control services in a variety of cropping systems.

Pest suppression by birds is best documented for tropical crops (e.g., Johnson et al. 2010 and Karp et al. 2013), particularly in coffee and other long-lived, perennial tree crops that are structurally more similar to native forests than are annual crops (Peisley et al. 2015; Boesing et al. 2017; Maas et al. 2019). In these perennial systems, birds have been shown to deliver pest control services that can scale up to greater profitability for farmers (Johnson et al. 2010; Karp et al. 2013). In temperate regions, however, studies tend to focus on crop damage which overlooks the potentially important role that birds play in controlling pests of vegetables and other annual crops (Peisley et al. 2015; Garfinkle et al. 2020). For example, Hooks et al. (2003) found that birds protected broccoli (*Brassica oleracea*) plantings in Hawaii, USA from damaging caterpillars. This translated into reduced foliar damage and greater productivity. In a similar study system looking at brassica crops in California, however, birds had no impact on kale damage or yield (Garfinkel et al. 2015). This neutral response was attributed to birds indiscriminately feeding on insect mesopredators in addition to pest insects. This highlights that like other generalist predators, birds often prey upon predatory arthropods in addition to herbivores (Martin et al. 2013). While it is clear that birds can both support and hinder biological control, the context governing which outcome will occur is unclear, particularly in temperate annual crop systems.

The potential cascading impacts of birds on crop damage and yield may be further complicated by seasonal dynamics (Pejchar et al. 2018). For example, Eurasian Tree Sparrows (*Passer montanus*) altered the types of arthropods they fed their chicks throughout the breeding season, with implications for provisioning of pest control services (Grass et al., 2017). During their first brood, the sparrows fed their chicks a diversity of insect families captured from a variety of habitats. However, during the second brood period, sparrows foraged more in annual crop fields and provisioned their young with more mesopredatory arthropods (e.g., hoverfly larvae and ladybird beetles). Finally, during the third brood period, sparrows increasingly fed their nestlings wheat and oat grains. These observations suggest birds switch diets throughout the growing season, potentially tracking changes in arthropod community dynamics, with subsequent differential effects of birds on pest predators and crop damage throughout the season (Grass et al. 2017).

Here, we examined how landscape context mediates the seasonal impact of birds on arthropod pests and natural enemies, leaf damage, and yields of broccoli on highly diversified farms managed by cooperating growers in Washington, Oregon, and California, USA (Figure 5.1). The primary pests of broccoli in the system include cabbage aphids (*Brevicoryne brassica* L.), green peach aphids (*Myzus persicae* Sulzer), diamondback moths (*Plutella xylostella* L.), cabbageworms (*Pieris rapae* L.), cabbage loopers (*Trichoplusia ni* Huebner), and crucifer flea beetles (*Phyllotretta crucifera* Goeze). The primary arthropod natural enemies include parasitoid wasps (largely *Diaeretiella rapae* Stary), lady beetles (Coleoptera: Coccinellidae), predatory syrphid fly larvae (Diptera: Syrphidae), spiders (Araneae), lacewings *(Chrysoperla* spp.), predatory wasps (Hymenoptera: Vespidae), and predatory bugs (*Nabis* spp., *Geocoris* spp and *Orius* spp. (Blubaugh et al. 2018,2021). Our study focused on highly diversified, mostly certified organic producers because of their greater reliance on biological control compared with more intensified systems (Smith et al. 2021). These organic farmers employ a variety of methods aimed to enhance biological control such as maintenance of hedgerows, interspersion of pollinator plantings that provide pollen and nectar to some natural enemies (e.g., syrphid flies and parasitoid wasps), nest boxes, and use of polyculture (Smith et al. 2021). However, flea beetles are primarily controlled by row covers and sticky traps in organic systems (Ekbom 2010). Farmers in our study infrequently used pesticides (Smith et al. 2021).

Our study had two complementary components. First, to identify arthropod prey in the diet of farmland bird species, we collected avian feces directly from birds captured in mist-nets (2016-2017) across our network of farms. Second, at a subset of these farms in Washington and Oregon, we conducted a bird exclusion experiment to determine the effect of birds on arthropod abundance, leaf damage, and broccoli yield. On each of the exclusion experiment farms, we excluded birds from feeding on a subset of broccoli plants, while allowing birds access to others. Our central hypothesis was that birds would have the greatest benefit to crop yield on farms embedded in the most natural landscapes via increases in pest arthropod consumption (Boesing et al. 2017). However, we also hypothesized that intraguild predation of predatory arthropods by birds could reverse or neutralize the effect of landscape context on natural pest control benefits and that seasonality (both of the annual season and crop growth stage) may play a role (Boesing et al. 2017, Karp et al 2018).

METHODS

Study Sites

Across 3 years (2016-2018), we conducted surveys to elucidate the net effects of birds on diversified crop production on farms throughout Washington, Oregon, and California, USA, previously described in Smith et al. (2020a,b, 2021). Farms grew a grange of crops including cereals (e.g., corn, wheat, barley), vegetables and melons (e.g., brassicas, leafy vegetables), fruits and nuts (e.g., citrus fruits, grapes, berries, walnuts), oilseed crops (e.g., olives, sunflower), roots (e.g., potatoes), spice crops (e.g., olives, sunflower), roots (e.g., potatoes), spice crops (e.g., chilies, peppers, fennel), beverage crops (e.g., tea), medicinal crops, commercial flowers, and grasses and fodder crops, among others. All farms grew brassica crops including broccoli and/or kale. Farms in the study were generally small scale (mean± SE = 25.3 ± 47.2 ha; range: 0.44–272.2 ha). We conducted mist-netting to obtain avian fecal samples for molecular diet analysis in 2016 and 2017 on 47 farms (Figure 5.1). In 2018, we recruited a subset consisting of 13 cooperating farms from Washington and Oregon to participate in our bird exclusion experiment. We asked all farms in the Pacific Northwest region to participate, and of those, 13 agreed. We limited our experiment to the Pacific Northwest (i.e. did not conduct the experiment in California) for feasibility.

Landscape context

To characterize landscape context, we calculated the percent natural/semi-natural (hereafter, “natural”) cover based on the 2016 National Land Cover Database (Homer et al. 2012) using a 1,000 m radius buffer centered at the cage location for our bird exclusion experiment and at the farm center for molecular diet analyses using Program R and FRAGSTATS 4.1 (McGarigal and Marks 1995). Natural cover included forest (deciduous, evergreen, and mixed), scrubland (dwarf scrub and shrub/scrub), herbaceous grassland/herbaceous, sedge/herbaceous, lichens and moss), and wetland categories (woody and emergent herbaceous wetlands). Categories not included in natural cover were water, ice/snow, developed, barren, pasture/hay, and cultivated crop classes. We used a 1,000 m buffer to encompass the daily activity centers for both arthropods and birds and because it is commonly used for arthropod assessments in agricultural systems (Lichtenberg et al. 2017, Billerman et al. 2020, Tamburini et al. 2020).

Bird Exclusion Experiments

At each of the 13 farms in our exclusion experiment, we established 3 bird exclosures and 3 controls placed consecutively in a randomized order, each surrounding 4 broccoli plants (Martin et al. 2013, Maas et al. 2019; Figure 5.2). In all cases, our experiment broccoli plantings were established within broccoli fields that had been planted by the cooperating growers between 28 June and 23 July 2018 (Figure 5.2D). We installed cages at the edges of plantings closest to semi-natural elements to control for possible variation in pest control services due to edge effects (Olimpi et al. 2020). Cages were installed between the day of planting and 6 days post-planting.

Cages were built from PVC pipe and covered in #6 monofilament 3.81-cm mesh netting (Figure 5.2C; Memphis Net & Twine, Memphis, TN, USA). Exclosures were 0.61-m tall and individualized in length and width to fit the crop spacing each grower used such that the cage would enclose 4 broccoli plants (range: 0.48 x 0.79 m to 1.2 x 1.2 m). Control plots were marked by thin twine strung on thing posts (~2-mm diameter) to discourage perching by birds (Maas et al. 2019).

We conducted mid-broccoli-growth (August 9-17) and late-broccoli-growth (September 7-23) surveys of arthropods (identity and abundance) and leaf damage (e.g., Karp et al. 2016). Visual arthropod surveys were conducted between 09:00 and 13:00 hours, always in absence of heavy rain (Blubaugh et al. 2018). Arthropods were counted on each plant and identified to the most detailed taxonomic resolution possible (e.g., lady beetle, aphid, parasitized aphid [hereafter, "aphid mummy"]). We did not collect arthropods to avoid altering community dynamics.

To measure leaf damage, we photographed one randomly selected leaf per plant per survey period. To randomly select leaves, we used the RAND function in Microsoft Excel to randomly assign a number between 0 and 1 for leaf 1 (closest to the ground) through the terminal leaf. We then sorted the random numbers and photographed the leaf number in the row containing the largest random number. We repeated the selection process for each of the 24 plants per farm. We photographed leaves attached to the plant against 1-cm graph paper in the mid-growth survey to minimize plant damage but removed and pressed leaves overnight prior to photographing in the late-growth survey when the plants were generally large and well established. Using the images, we calculated the percent of leaf damaged using the software ImageJ (Abramoff et al. 2004, Kalka et al. 2008). We estimated crop yields by cutting all mature heads 14 cm below the crown height following the standard of farms in the study and immediately measured mass in grams. Heads were harvested from 26 August to 21 October 2018, depending on the variety planted, grower management, and microclimate of experimental plots.

We examined the impacts of bird exclusion (i.e. treatment), survey period (mid-growth vs late-growth), and percent natural cover in the landscape (1,000 m) on (1) crop pest abundance (aphids, caterpillars, and flea beetles), (2) arthropod natural enemy abundance (aphid mummies, all predators, spiders, and syrphid larvae), and (3) leaf damage using generalized linear mixed effects models via the *glmmTMB* package in R (Magnusson et al. 2017). We summed arthropods across the 4 plants per plot and used plot as the unit of replication in our arthropod analyses. We included plot nested within farm as a random effect in all models. Models examining abundance used a negative binomial distribution, and models examining leaf damage used a betabinomial distribution, both to account for overdispersion in the data. To examine the importance of bird exclusion, survey period, and percent natural cover in the landscape, we compared all nested models from our global model that included their 3-way interaction. We constructed a completed model set consisting of an intercept-only null model as well as all single variable, additive, and interactive models with treatment, % natural, and survey period variables as fixed effects. All 15 models in our set included plot nested within farm as a random effect.

We compared models based on Akaike Information Criterion corrected for small sample sizes (AIC_c) in the *bbmle* package in R (Bolker 2021) and identified the top-competing models as those within $\Delta AIC_c < 2.0$ of the most well-supported model (Anderson et al. 2001, Burnham and Anderson 2002). We then estimated covariate effects by model averaging among the best-supported models (within 2 ΔAIC_c of the best-supported model) using the *model.avg* function in the *MuMIn* package in R (Burhnham and Anderson 2002, Barton 2020). We considered covariates as strong predictors of the response variables if they appeared in the top models ($\Delta AIC_c < 2.0$) and their model-averaged 95% confidence intervals did not overlap zero.

To compare broccoli head weight ("yield") between treatments, we used the log-response ratio as an effect size metric (Hedges et al. 1999). We averaged the head weight across plants within treatments within farms and calculated the effect size by talking the natural log of the average head wight of controls, divided by the average exclosure plot head weight. We used the log-response ratio rather than the absolute head weight per plot to account for variation in crown weight between varieties grown by different farms. This is because varieties can differ greatly in weight (min = 53 g ["broccolini" variety] to max = 356 ["gypsy" variety]). We installed cages within commercial fields planted by cooperating farms, so we were unable to mandate that all growers plant the same variety. We used a two-tailed one sample *t*-test to determine whether the mean head weight differed between bird exclosure and bird access plots.

Molecular Diet Analysis

We collected fresh fecal samples from mist-netted birds for molecular diet analysis. We visited farms in a south–north transect and generally visited each farm twice per year, but variation occurred due to weather and grower schedules. In total, we collected 2042 feces from 2024 birds from 76 species captured on 47 farms between 27 April and 18 September 2016 and 3 May and 29 Aug 2017 (Figure 5.1A and 5.2D) We note that the exclosure experiment arthropod survey dates coincide with the "late" mist-net season and occurred the following year (2018). This was due to logistical constraints in our ability to conduct the experiment and netting concurrently and to maximize the number of farms able to participate in the exclusion experiment in the study region where the peak transplant period is late-June/early-July.

From the 2,024 birds captured through mist-netting, we selected 5 species for molecular diet analyses (American Goldfinch [*Spinus tristis*] American Robin [*Turdus migratorius*], Savannah Sparrow [*Passerculus sandwichensis*], Song Sparrow [*Melospiza melodia*], and White-crowned Sparrow [*Zonotrichia leucophrys*]). We selected these species because they were commonly observed foraging in broccoli plantings (Smith et al., 2020a,2022) and had sufficient numbers (≥ 50) of fecal samples to model variation across farms (Taylor et al 2022 Dryad Dataset S1). Between the 5 focal species, we had 357 fecal samples from birds captured on 34 of the farms. The number of samples per species ranged from 54 (American Robin) to 103 (White-crowned Sparrow). Our choice of focal species was constrained by relatively low captures per bird species (see Taylor et al 2022 Dryad Dataset S1for full details on number of samples per bird species). Thus, we included all species with >50 fecal samples in our diet analyses, provided that they were also observed foraging in brassica crops. To determine if species foraged in brassica crops, we conducted formal point count surveys from 2016 to 2019 described in Smith et al. (2020a, 2022). In addition, individual birds observed using broccoli fields during the experiment setup, arthropod surveys, and yield surveys were identified and recorded (Taylor et al 2022 Dryad Dataset S1).

Our mist-net protocol was previously described in detail in Smith et al. (2020a). Briefly, we placed 4–8 mist-nets around farms in locations selected to maximize capture rates and moved nets if capture rates were low and high activity was noted in another location. We occasionally placed nets immediately adjacent to each other to create longer nets along habitats with high bird abundances. Captured birds were placed in cloth bags that were washed after use, given unique leg bands, weighed, and measured. Feces were placed in 200 proof ethanol in cryotubes and immediately stored in a liquid nitrogen shipment tank until shipment to Washington State University on dry ice. Samples were stored at -80ºC until DNA extraction.

To extract prey DNA from avian feces, we used QIAamp DNA stool mini kits (Qiagen, Hilden, Germany) following the manufacturer's protocol with modifications to increase DNA yields described in Supplementary Methods of Smith et al. (2020a). Each bird sample was then PCR amplified for arthropod-specific cytochrome c oxidase I (CO1) regions using the ZBJ-ArtF1c (F) (AGATATTGGAAC*TTATATTTTATTTTTGG) primer set (Zeale et al. 2011). Arthropod primers were used with the following protocol: 95ºC for 5 min, followed by a touchdown protocol of 16 cycles of 94ºC for 30s, 61ºC (decreasing 0.5ºC cycle^{-1}) for 30 s and 72ºC for 30 s, then 17 cycles of 94ºC for 30 s, 53ºC for 30 s and 72ºC for 30 s followed by a final extension at 72ºC for 10 min. PCR products were cleaned using AMPure beads following a modification of Rohland and Reich (2012), then pooled for each sample. Pooled samples were then prepared for Illumina sequencing following the protocol for NEBNextUltra II DNA Library Prep Kit for Illumina. After end repair and adaptor ligation, we again cleaned amplicons using AMPure beads. We then used NEBNext Multiplex Oligos for Illumina Index Primer Sets 1 and 2 for primer enrichment. All samples were cleaned a final time using AMPure beads, quantified using Qubit Fluorometer (ThermoFisher Scientific) to quantify the DNA concentration of each library, then pooled and submitted for sequencing on the Illumina MiSeq platform (2 x 300 bp) at the University of California Riverside Institute for Integrated Genome Biology. We included negative controls during the sequencing process to detect contamination or sequencing errors but did not detect any issues.

Demultiplexed sequences were processed using the Anacapa Toolkit (Curd et al. 2018, Curd and Ogden 2018). First sequences were trimmed using *cutadapt* (Martin 2011) and quality sorted using the *fastx*-toolkit (Gordon and Hannon 2010). *dada2* (Callahan et al. 2016) was used for denoising, dereplicating, merging and removing any chimeric sequences. Taxonomy was assigned using Bowtie 2 (Langmead and Salzberg 2012) and a Bayesian Least Common Ancestor algorithm. For each bird, we then assessed the total number of reads for each amplicon sequence variants (ASV) summarized at a bootstrap confidence of 95.

Because the number of reads does not always correlate to the amount of tissue of a specific diet item in a sample (Bell et al. 2019, Guenuning et al. 2019), we analyzed the percentage of fecal samples that contained DNA from various prey items. We restricted analyses to samples with at least 100 identified reads and normalized sequenced read counts by dividing the number of reads per amplicon sequence variant (ASV) by the total number of species-level identified ASVs assigned to phyla Arthropoda and Mollusca. We considered species to be present if they represented at least 1% of the reads per sample and at least 5 reads per ASV (Garfinkel et al. 2020). Prey items were identified as either brassica pests (*Plutella xyllostella*, *Trichoplusia ni*, *Pieris rapae*, *Phyllotrea striolata*, *Brevicoryne brassicae*, and *Myzus persicae*) or natural enemies observed to be present on farms in our study and/or on farms in other studies done in similar systems (Blubaugh et al. 2018, 2021). In addition, while pest sequences were identified to the species level, we conducted our analyses at the family level (i.e. Plutellidae, Noctuidae, Pieridae, Chrysomelidae, and Aphididae) to align with natural enemies. We had to aggregate natural enemies at the family level due to our inability to accurately verify species-specific presence of these taxa in our system. Other taxa detected (e.g., detritivores or other species not known to be brassica pests or arthropod natural enemies of brassica pests) were excluded from our analysis.

Predation pressure on trophic groups was calculated by assigning detected prey species as either in the family of known natural enemies of brassica pests (predator and parasitoids) or in the family of known brassica pest species. We examined the impacts of bird species, season (late spring/early summer survey or late summer/early fall survey), and percent semi-natural cover in the landscape on the likelihood of individual birds having (1) arthropod natural enemy taxa and (2) brassica pest taxa detected in their fecal samples. To do so, we used generalized linear mixed effects models with a binomial distribution via the *glmmTMB* package in R (Magnusson et al. 2017). We included farm as a random effect in all models. Models were run using the Bobyqa optimizer. We repeated the model selection approach described above for our experiment. We constructed a complete model set consisting of an intercept-only null model as well as all single variable, additive, and interactive models with species, % natural, and season variabilities as fixed effects. All 14 models in our set included farm as a random effect.

For these models, we assessed if variables improved model fit using likelihood ratio tests (due to species being a categorical variable) for all models with weights > 0.05. We used generalized Tukey HSD tests in the *multcomp* package in R (Hothorn et al. 2008) to examine differences in categorical predictor variables that had high support (were included in models with $< 2\ \Delta AIC_c$) and improved model fit (likelihood ratio tests).

RESULTS

Bird Exclusion Experiment

Aphid abundance was higher in the presence of birds (model averaged β: 0.92, 95% CI: 0.079–1.76) and in the late-growth survey (model averaged β: 2.59, 95% CI: 2.25–2.92) but was not strongly impacted by landscape (Figure 5.3A; Appendix Table 5). Caterpillar abundance, in contrast, was not strongly impacted by the bird exclusion treatment (model averaged β: −0.15, 95% CI:-0.55 to 0.24), landscape (model averaged β: 0.0035, 95% CI: -0.0044 to 0.011), or survey period (model averaged β: -0.056, 95% CI: -0.33 to 0.22), although caterpillars tended to be more abundant in bird exclosure plots (Figure 5.3B; Appendix Table 6). We found no effect of bird exclosure treatment on flea beetle abundances (Figure 5.3C; Appendix Table 7). However, flea beetle abundances tended to be higher in the least natural landscapes during the mid-growth survey but were generally low across all landscapes in the second survey (model averaged β: 0.11, 95% CI: 0.072-0.15).

In alignment with aphid abundance, aphid mummy abundance was higher in the presence of birds (model averaged β: 1.00, 95% CI: 0.048-1.95) and in the late-growth survey (model averaged β: 1.37, 95% CI: 0.98-1.76) but did not have a strong relationship with percent natural cover in the landscape (Figure 5.4A; Appendix Table 8). However, when considering the proportion of aphids that were parasitized, mummies were proportionally higher during the mid-growth survey compared to the late-growth survey (model averaged β: -0.95, 95% CI: -1.31 to -0.59), and bird exclusion had a weak effect (model averaged β: 0.22, 95% CI: -0.21 to 0.64) (Figure 5.4B; Appendix Table 9). Predatory arthropods were more abundant in the late-growth survey than the mid-growth survey (model averaged β: 1.16, 95% CI: 0.71-1.61) but were not strongly impacted by bird exclusion or landscape context (Figure 5.4C; Appendix Table 10). The most abundant predators (spiders and syrphid fly larvae) had differing responses to landscape context in the mid-growth survey: spiders were more abundant in the least natural landscapes (model averaged β: -0.018, 95% CI: -0.032 to -0.0037) but appeared consistently across the landscape gradient in the late-growth survey (model averaged β: 0.017, 95% CI: 0.0016-0.033) (Figure 4D; Appendix Table 11). In contrast, syrphid larvae were not strongly impact by landscape (model averaged β: 0.012, 95% CI: ⁻0.0066 to 0.030), and similar to most arthropod groups, were more abundant in the late-growth survey (model averaged β: 1.80, 95% 0.95⁻2.64) (Figure 5.4E; Appendix Table 12). Bird exclusion did not strongly impact leaf damage, but leaf damage was higher in less natural landscapes (model averaged β: ⁻0.016, 95% CI: ⁻0.024 to ⁻0.0073) and in the mid-growth survey (model averaged β: ⁻0.39, 95% CI: ⁻0.69 to ⁻0.085) (Figure 5.5A; Appendix Table 13). The broccoli head weight response ratio was not significantly different than 0, indicating no significant impact of bird exclusion on yield (one-sample *t*-test, $t = 0.84$, $P = 0.42$). The broccoli head weight response ratio did not vary across the landscape gradient (Figure 5.5B; β = ⁻0.0008 ± 0.004, $P = 0.85$).

Molecular Diet Analysis

Percent natural cover in the landscape was a weak predictor of both arthropod natural enemy and pest detections in avian feces (Appendix Table 14). However, season of capture was a good predictor for both: birds captured late in the growing season (late summer/early fall (August -September) were less likely to have natural enemies detected in their feces (Table 5.1; odds ratio = 0.64, 95% CI = 0.39–1.05). Conversely, the odds of detecting pest DNA in their feces was two times higher in the late season (Table 5.2; odds ratio = 2.06, 95% CI = 1.33–3.21). Bird species identity was a strong predictor of the detection of arthropod natural enemy, but not pest, taxa (Appendix Table 14-15). White-crowned Sparrow feces were more likely to contain arthropod natural enemy DNA than American Goldfinches (Table 5.1; odds ratio = 2.84, 95% CI = 1.42–5.69). At the family level, arthropod natural enemies were detected in a sizable minority of samples (Bipartite analysis; Figure 5.6), with 22.0%, 17.7% and 10.5% of all birds sampled having detections of carabid beetles, syrphid flies, and braconid wasps, respectively (Table 5.3). Pest families were detected at similar levels with 22.4%, 11.5%, 16%, 0.56% and 4.48% of birds having detections of Aphididae, Chrysomelidae, Noctuidae, Pieridae, and Plutellidae respectively (Figure 5.6; Table 5.4). However, species level detections of known pests were much rarer with only 4.48%, 4.2%, 1.4%, 0.56%, 0.28%, and 0.28% of sampled birds having detections of *P. xylostella*, *M. persicae*, *P. striolata*, *B. brassicae*, *T. ni*, and *P. rapae,* respectively (Appendix Table 16).

DISCUSSION

Our bird-exclusion experiment suggested that birds have relatively modest impacts on the arthropods associated with broccoli plantings. Bird access yielded higher, rather than lower, numbers of pest aphids (Figure 5.3A) and increased the number of parasitized aphids (Figure 5.4A). That is, access by birds to the arthropod community appeared to result in a net increase, rather than decrease, of aphid abundance and aphids' specialist natural enemies. In contrast, we did not detect a strong change in the numbers of caterpillars nor flea beetles on broccoli plants accessible to birds versus those where birds were excluded (Figure 5.3B-C). This could be because birds infrequently preyed upon these pests in the mid-to-late growing season, or because birds ate a mix of these pests and their natural enemies, such that the direct and indirect effects were counteracting (Mooney and Linhart 2006). We found weak impacts of birds on densities of predatory arthropods (Figure 5.4C-E). Any impacts of birds on arthropod communities, however, did not translate into strong leaf damage nor yield differences between treatments (Figure 5.5).

Our molecular diet analysis highlighted the complex dynamic of bird foraging on farms. Each of the 5 focal bird species examined had at least 1 brassica pest species occurrence in their diets. At the same time, arthropod predators of these pests were also likely to be detected (Figure 5.6). Landscape context did not strongly impact the likelihood of detecting pests or enemies in bird diets. Yet, we found some evidence for bird species differing in propensity for intraguild predation (Figure 5.6), and prior work has shown that farm bird community composition shifts along landscape gradients (Smith et al. 2020a, 2020b, Olimpi et al. 2020). For example, we have previously shown that bird community composition in brassica fields on the same or similar farms in California, Oregon, and Washington shifts across the gradient of percent semi-natural cover (Smith et al. 2020a). Additionally, we have shown these same farms embedded in more natural landscapes have lower nonnative bird densities (Smith et al. 2020a). The response of native species density and richness to landscape context was more nuanced with an interaction observed between local diversification through crop-livestock integration and percent natural cover in landscape (Smith et al. 2020a). Recent analyses using point count data from farms only in Oregon and Washington (where our experiment was conducted) found lower overall bird abundances on farms in more natural landscapes, alongside reduced ecosystem disservice provider indices (Smith et al. 2022). Therefore, changes in landscape is likely to lead to different bird assemblages, which may lead to differing degrees of intraguild predation and variations in food web interactions along landscape gradients.

In addition to known effects of landscape composition on bird communities in our system (Smith et al. 2020a, 2020b, 2021), our findings demonstrated contrasting impacts of landscape context on several key arthropod pest and enemy groups. Flea beetles decreased with increasing natural cover in the mid-growth survey, with this relationship disappearing by the late season as the flea beetle densities fell across all farms (Figure 5.3C). In contrast, we observed a weak tendency towards higher numbers of syrphid larvae in fields surrounded by a higher proportion of natural cover and in the late-growth survey. Because we were counting syrphid larvae per plot, this implies higher oviposition, which may increase with greater floral resources that can support a large adult population (Burgio et al 2015). Spider densities, however, were higher in the mid-growth survey in less natural landscapes, though this pattern disappeared later in the growing season. We hypothesize this may be due to increased immigration from semi-natural areas into fields as brassica plants matured, providing more structure for spiders later in the growing season. Another reason could be that spiders play a vital role in the early development of many passerines, the provisioning of which coincides with mid-growth surveys (Ramsay and Houston 2003; Arnold et al. 2007). In support of this, previous studies have demonstrated an increase in foliage-gleaning insectivores in more natural landscapes (Barbaro et al. 2017; Olimpi et al. 2020), suggesting that insectivorous birds in natural landscapes may have prized spiders on plants in the mid-growth surveys when the birds were still provisioning chicks.

We observed a decrease in leaf damage on farms in more natural landscapes and in the late-growth survey (Figure 5.5A). The leaf damage results primarily tracked the flea beetle population trends. Flea beetles are primarily controlled by row covers and sticky traps in organic systems rather than by natural enemies (Ekbom 2010). The importance of flea beetle damage during the study period may have masked the impacts of other brassica pests (caterpillars, aphids) that do have effective natural enemies (Hooks et al. 2003). Despite the negative impacts of bird exclusion on aphid abundances and parasitism rates, we did not observe any impact of bird exclusion on eventual broccoli head weight (Figure 5.5B). Prior work has noted attenuation of top-down effects wherein impacts of natural enemies on pests may not scale to variation in crop damage or yield (Schmitz et al. 2000, Mäntylä et al. 2011, Kleijn et al. 2019). This may especially be the case for plants with antiherbivore defenses like broccoli (Schmitz et al. 2000, Blubaugh et al. 2018). This might be particularly true when crop pests do not directly damage the harvestable portion of the crop, as is the case with broccoli in which pests often attack leaves but not the broccoli head. Additionally, compensatory growth and regeneration may outweigh the impacts of biocontrol (Lemesa et al. 2015, Liere et al. 2015). Finally, we note that broccoli plants are most vulnerable to leaf damage as seedlings when herbivory can be fatal, but vulnerability to herbivory diminishes as plants age (Lamb 1989). Therefore, biocontrol agents may be most beneficial at younger plant stages.

A large body of research has examined the relationship between landscape structure, particularly the proportion composed of agricultural versus natural cover, on biotic abundance and biodiversity in crop fields (Chaplin-Kramer et al. 2011; Karp et al. 2018). However, results have been highly inconsistent, and a recent meta-analysis found no consistent impacts of landscape composition on pest and enemy abundances, predation rates, crop damage, or yields (Karp et al. 2018). Several hypotheses have been proposed for this inconsistency including pest populations not having effective natural enemies in the region; natural cover being a greater source of pests than natural enemies; crops providing more resources for natural enemies than natural cover; natural cover being insufficient in amount, proximity, composition, or configuration to provide large enough enemy populations needed for pest control; and agricultural practices counteracting enemy establishment and biocontrol provided by natural cover (Tscharntke et al. 2016). Additionally, differences in the ecology of key pests and natural enemies between crop systems may lead to high variability in outcomes and strong context-dependency (Karp et al. 2018). We suggest that another reason for inconsistent findings could be that birds, and perhaps other intraguild predators, could cause further idiosyncrasy of landscape effects on pests and crop damage (Martin et al. 2013). Between our bird exclusion experiment and molecular diet analysis, we found that birds were exerting a complex mix of direct predation on pests and arthropod natural enemies, with the former possibly counterbalanced by the latter. Additionally, the natural enemies themselves sometimes exhibited higher, but other times lower, abundances in landscapes with more natural cover. The combination of species-specific responses to landscape structure, each with complex feeding roles that do not easily fit into clearly defined trophic levels, might be preventing the generation of simple, consistent relationships between landscape structure and biocontrol. This may be particularly the case if avian and arthropod natural enemies can substitute each other and form a complex reticulate food web (Mooney and Linhart 2006).

Limitations and future directions

We note several limitations to our study. First, the exact arthropod natural enemy/pest ratio in bird diets is difficult to interpret. Furthermore, the magnitude of the predation pressure is not clear from our molecular analysis alone. Additionally, while brassica pests are likely restricted to the crop fields, arthropod natural enemy taxa could have been encountered by birds in surrounding non-crop areas as well. Highly mobile bird species may have consumed enemies in crop fields or in semi-natural elements around the farm and in the broader landscape. Indeed, our prior point count surveys have documented high use of seminatural elements around fields by many bird species (Smith et al. 2020a). Many of the bird species that we observed to use brassica fields during our surveys have home ranges or daily movements large enough to cover the relatively small-scale farms in our study (mean farm size ± SE = 25.3 ± 47.2 ha, range: 0.44–272.2 ha). The average field size in which we installed exclusion cages was just 1.92 ± 0.46 ha (range: 0.058–5.68 ha). In comparison, Passerellidae sparrows that are some of the most common foragers on broccoli (Smith et al. 2020a) and that we included in our diet analysis have average home ranges that vary from ~3.4 ha (Song Sparrow) to 20 ha (White-crowned Sparrow) (Billerman et al. 2020; Supplementary Material Dataset S1). Thus, birds likely move throughout farms and between crop fields and seminatural elements where they may consume arthropod prey.

Additionally, we note that we were constrained in our choice of focal species by those that were commonly captured (Supplementary Material Dataset S1), although we did choose species that were commonly observed foraging on our focal crop (Smith et al. 2020a, 2022). In particular, we were unable to examine the diet of some highly insectivorous species that foraged on our focal crop (e.g., Common Yellowthroat [*Geothlypis trichas*]). The species examined in our diet analysis are generally considered either omnivorous (American Robin, Savannah Sparrow, and Song Sparrow) or granivorous (American Goldfinch and White-crowned Sparrow) during the breeding season (Wilman et al. 2014, Billerman et al. 2020). However, recent molecular diet analysis is revealing that species typically thought to be consistent granivores may have greater degrees of insectivory than previously thought (Garfinkel et al. 2021), which our results may support in that we observed arthropods such as aphids in American Goldfinch diets (Table 5.4; Supplementary Material Table S12). We also note that these omnivorous/granivorous species may provide weed control services (Brennan 2020) that were beyond the scope of this study.

A limitation of our experiment is that we are uncertain of how birds behaved on control plants. Future work could conduct focal observations or use camera traps paired with sentinel prey to measure predation frequency (Milligan et al. 2016). Additionally, our yield measurement was limited to head weight rather than marketable yield. Plants that had sustained heavy herbivory often had bitter broccoli heads, which may have reduced the quantity actually sold to customers (J.M.T. and O.M.S., personal observation). Future work should investigate crop quality changes in relation to bird foraging impacts and arthropod damage. Other brassica crops in which the pests directly damage the harvestable portion of the crop, such as kale, might be more strongly impacted by bird and arthropod natural enemies.

Finally, our results suggested that there were seasonal differences in the impacts of birds and arthropods, both tracking the calendar season and broccoli growth stage. A growing body of work has demonstrated the importance of seasonality on the diet of birds and subsequent pest control service provisioning or disruption (e.g., Grass et al. 2017, Rusch et al. 2017, McClenaghan et al. 2019). We began our exclusion experiment at the end of June to coincide with when the most farms would be growing broccoli (Figure 2D). However, we hypothesize that if we had begun our experiment at the start of the avian breeding season that we would have observed greater biocontrol when birds more heavily provision young with lepidopterans (Grass et al. 2017, Pejchar et al. 2018). In contrast, our late-growth survey occurred in September when many bird species shift diets towards plant material and may have more limited impacts on arthropod populations (Nyffeler et al. 2018). We hypothesize that biocontrol potential by birds would be highest in brassica crops if they are mature during the chick-rearing phase of the avian annual cycle, coinciding with the “spring/early summer” mist-net survey. Brassica production systems would provide a unique opportunity to test this hypothesis because they are grown and harvested year-round in many places, including the Pacific Northwest region. There may also be annual variation in pest control efficacy and bird diets tracking various factors such as pest outbreaks or environmental variability (Liere et al. 2015, McClenaghan et al. 2019).

Conclusion

The need to feed a growing human population, while also conserving birds and other sensitive species, is increasing interest in promoting farming practices that balance food production with biodiversity preservation (DeFries et al. 2004, United Nations 2020). This is easiest when wildlife clearly contributes to natural pest control or provide other ecosystem services that benefit farmers (Smith et al. 2021). Our findings suggest that growers may not experience large benefits from birds on crop pest control in all systems. Indeed, a recent meta-analysis by Diaz-Siefer et al. (2021) found an overall positive effect of birds on pest control but with high context dependency between crop types and farming systems. This suggests that additional financial incentives may be needed to encourage farmers to preserve natural elements or engage in other bird-friendly behaviors (e.g., installing nest boxes) that benefit bird conservation.

For example, farmers can enroll in private-sector or NGO-led eco-certification programs (e.g., Audubon Certified Grazed on BirdFriendly Land and Smithsonian Bird Friendly coffee) that enable them to sell their products at a price premium (Biggs et al. 2021). Additionally, although not widely adopted, there are some policies in place in certain regions that can promote landscape-scale restoration and management that could also benefit bird conservation e.g., landscape-wide implementation of agri-environment schemes in Europe (Dallimer et al. 2010, Santos et al. 2021). Altogether, greater implementation of bird-friendly practices in agricultural systems are urgently needed to halt the rapid declines of birds (Rosenberg 2019), and greater incentives are likely needed for farmers to do so.

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Figures

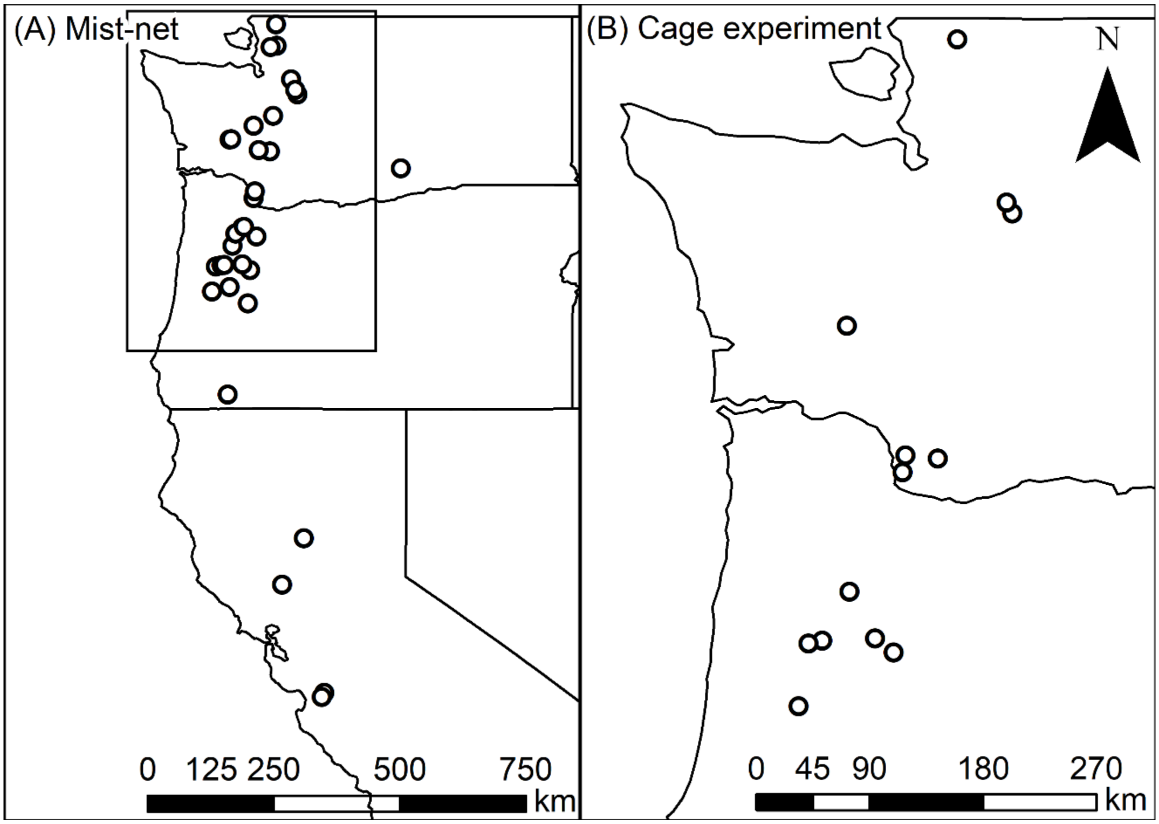


Figure 5.1. Map of farms included in our study. Includes **(A)** mist-netting locations (n=34) from which we collected fecal samples from focal species used in our study and **(B)** our bird exclusion experiment (n = 13). The box in **(A)** denotes where **(B)** falls within **(A)**.

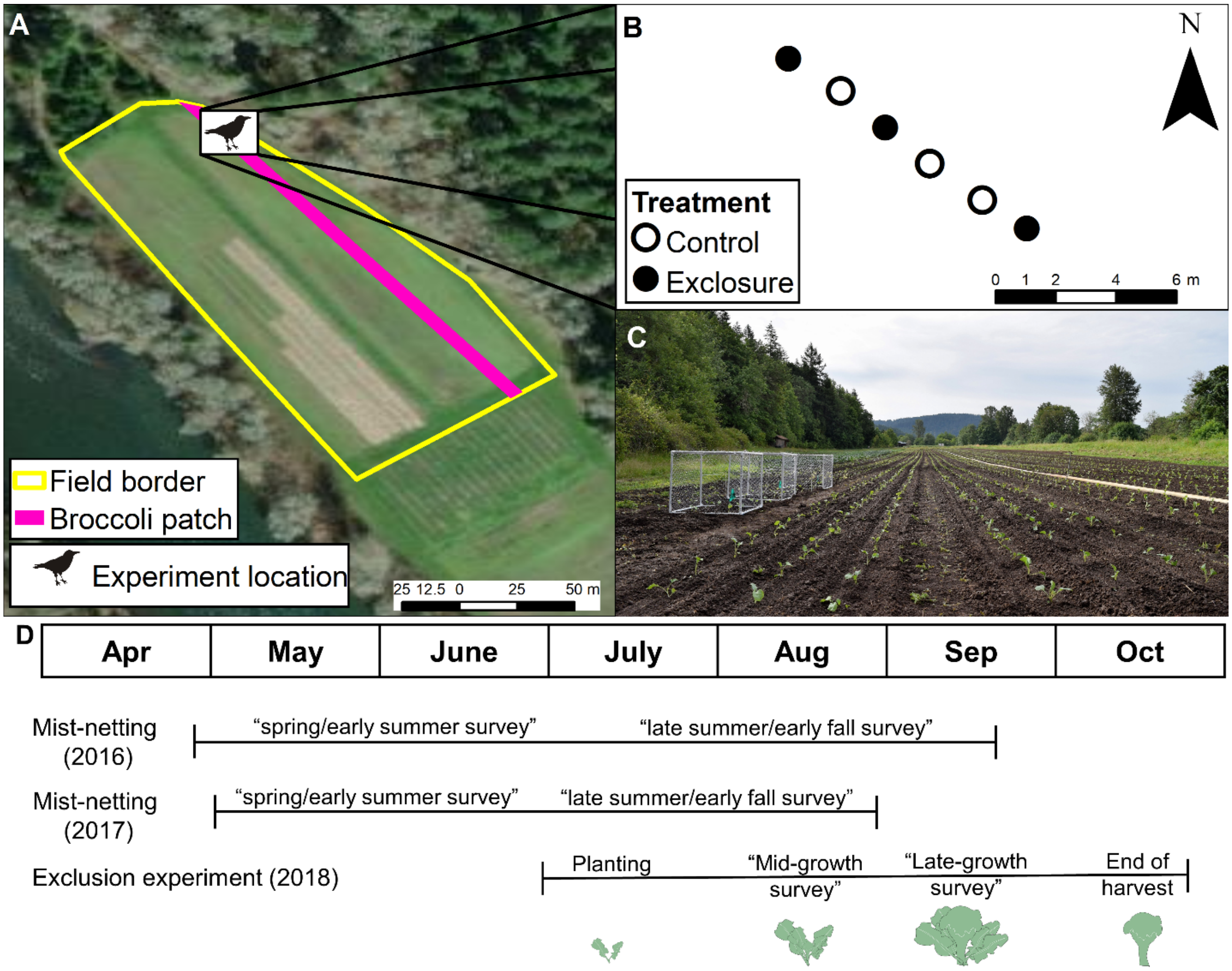


Figure 5.2. Schematic of a field from the bird exclusion experiment and research timeline. **(A)** Schematic showing where the exclosure (bird icon) was placed in relation to the broccoli patch (pink rectangle) and entire field (yellow border). Fields are defined as contiguous cropping areas bordered by grassy (non-cropped) margins, semi-natural margins, or roads. **(B)** Inset shows the randomized order in which treatments were placed. **(C)** Photo showing the exclusion experiment shortly after cage installation. **(D)** Timeline showing the overlap between the mist-netting "seasons" and bird exclusion experiment surveys. Photo credit: Olivia Smith.

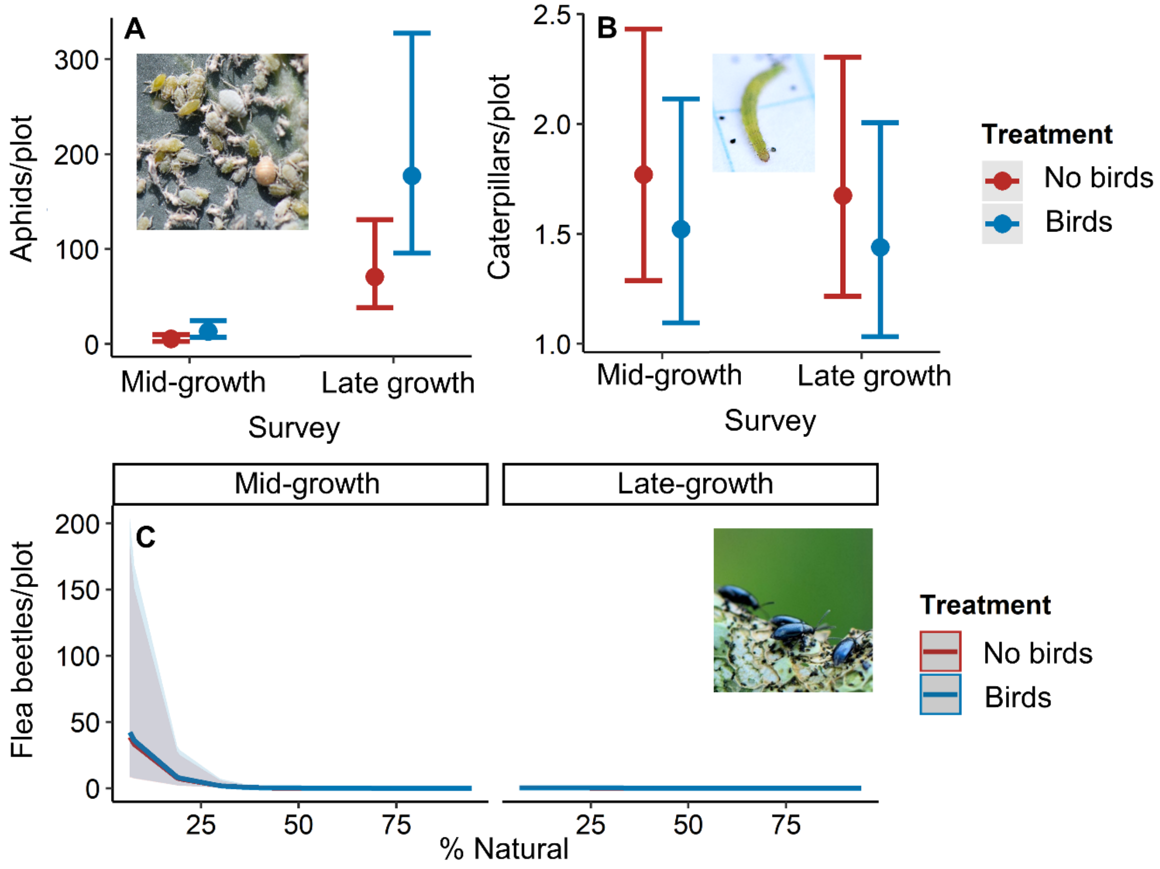


Figure 5.3. Impact of bird access/exclusion, % natural cover (1,000 m), and survey period on brassica pests. **(A)** Number of aphids and **(B)** caterpillars per plot in both mid-growth and late-growth surveys by bird access/exclusion. **(C)** Interaction between flea beetles detected per plot and % natural cover (1,000 m) and bird access/exclusion. The best-supported models that included bird exclusion treatment are plotted for each (see Supplementary Material Tables S1-S3). Photo credit: Olivia Smith.

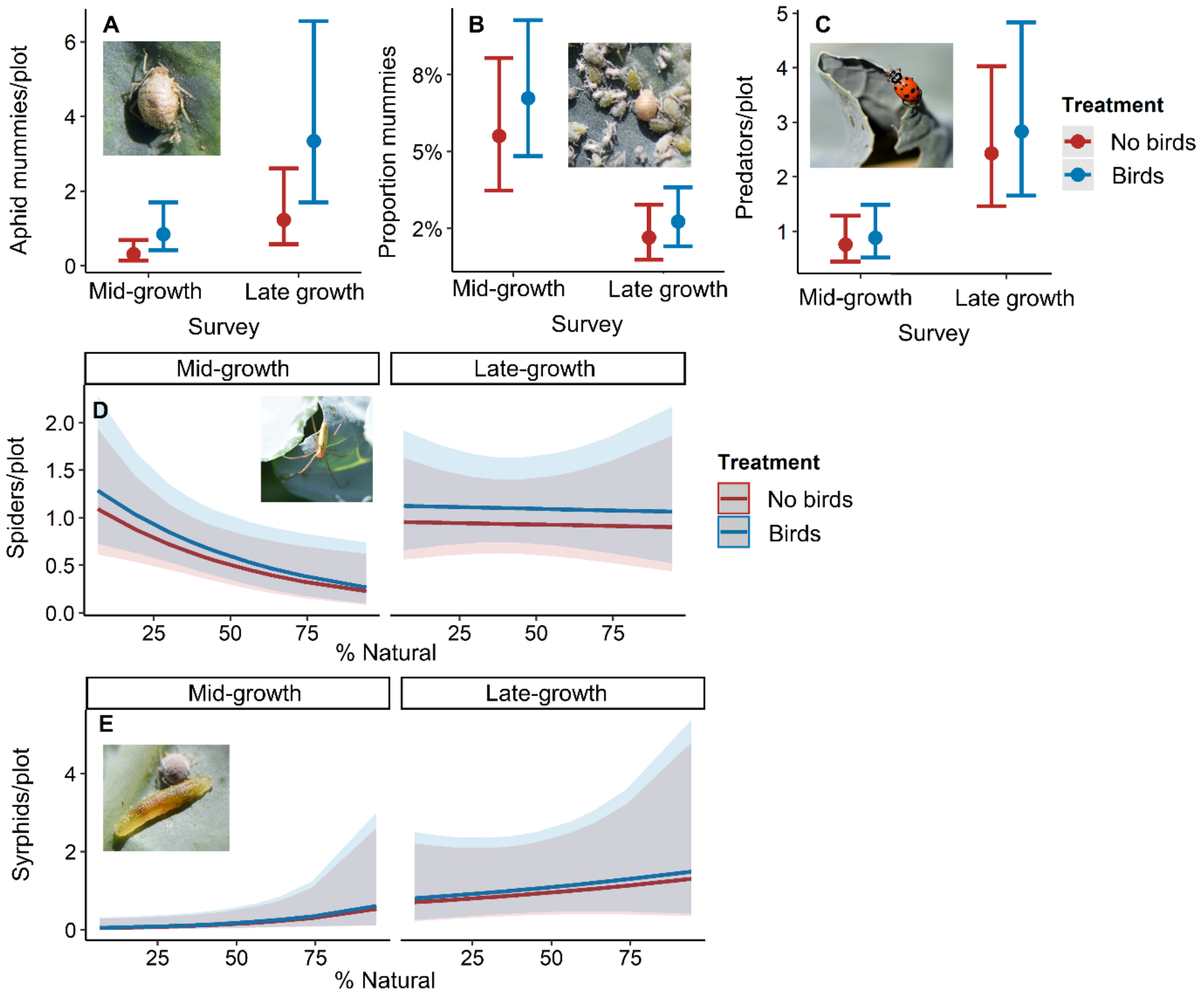


Figure 5.4. Impact of bird access/exclusion, % natural cover (1,000 m) and survey period on arthropod natural enemies of brassica pests. (A) the number and (B) proportion of parasitized aphids (mummies) counted per plot in both mid-growth and late-growth surveys. (C) Total number arthropod predators detected per plot. (D-E) Interaction between (D) spiders and € syrphid larvae detected per plot vs. % natural cover (1,000 m) in both the mid-growth and late-growth surveys. The best-supported models that included bird exclusion treatment are plotted for each (see Supplementary Material Tables S4-S8). Photo credit: Oliva Smith.

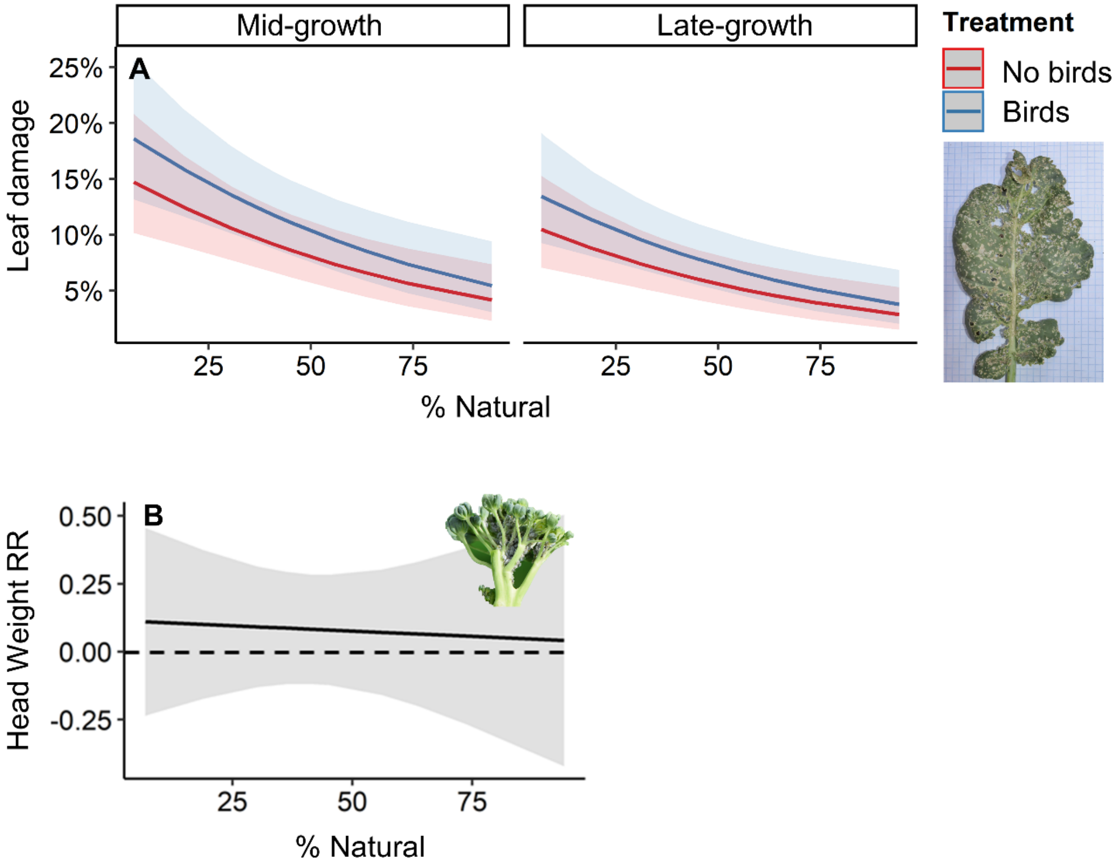


Figure 5.5. (A) % of leaf damaged vs % natural cover (1,000 m) in both mid-growth and late-growth surveys. The best-supported model that also included bird treatment is plotted (fixed effects of % natural, survey period, and treatment; see Supplementary Material Table S9). Photo shows a leaf with heavy flea beetle damage. (B) Effect size (log-response ratio = RR) of bird access vs exclosure on broccoli head weight vs. % natural cover (1,000 m; model only includes % natural cover). Photo credit: Olivia Smith.

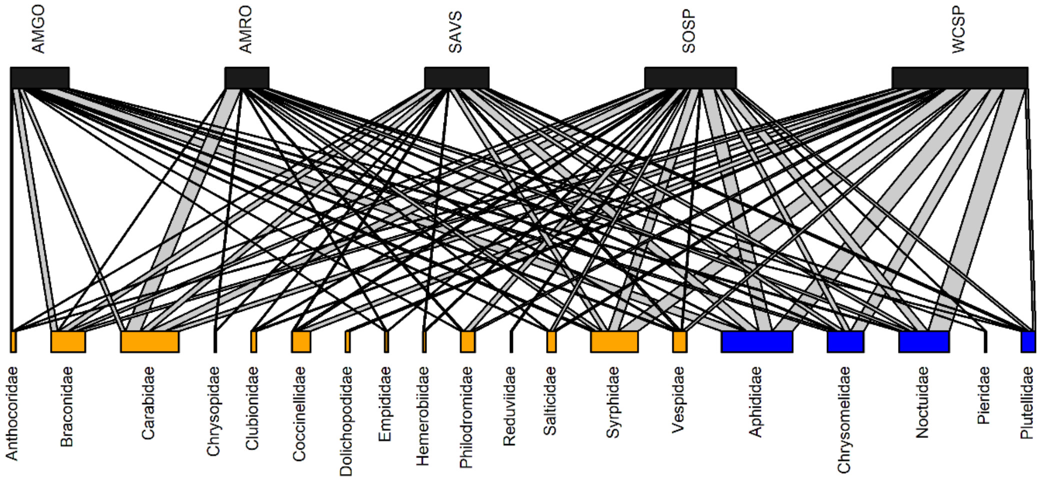


Figure 5.6. Bipartite graph showing feeding relationships between focal bird species and arthropod natural enemies (orange) and brassica pests (blue). Each box at the base of the figure represents an arthropod taxon (natural enemies = orange, brassica pests = blue), and the black boxes at the top represent the bird species. Rectangle size and gray line width are proportionate to the number of detections. Arthropods were analyzed at the family level. AMGO = American Goldfinch, AMRO = American Robin, SAVS = Savannah Sparrow, SOSP = Song Sparrow, WCSP = White-crowned Sparrow.

Tables

Table 5.1. Logistic regression table for arthropod natural enemy detections in bird feces from the best-supported model including the fixed effects of season and bird species (Natural Enemies ~ Bird Species + Season + (1|Farm)). The intercept comparison for this model is AMGO during mid-season. See Supplementary Material Table S10 for AIC$_c$ comparisons of models.

		Natural Enemies		
Predictors		Odds Ratio	CI	p
(Intercept)		0.60	0.30-1.20	0.148
Species [AMRO]		1.66	0.73-3.80	0.227
Species [SAVS]		1.94	0.89-4.21	0.096
Species [SOSP]		2.08	1.00-4.33	0.051
Species [WCSP]		2.84	1.42-5.69	**0.003**
Season [Late]		0.64	0.39-1.05	0.077
Random Effects				
σ^2		3.29		
$^{\tau}$00 Farm		0.04		
ICC		0.01		
^{N} Farm	31			
Observations	357			
Marginal R^2 /Conditional R^2	0.053/0.064			

Table 5.2 Logistic regression table for brassica pest detections in bird feces from the best-supported model including the fixed-effect of season alone and random effect of farm (Brassica pest ~ Season + (1|Farm)). The intercept comparison is against mid-season. See Supplementary Material Table S10 for AIC_c comparison of models.

		Brassicae Pests		
Predictors		Odds Ratio	CI	p
(Intercept)		0.49	0.34-0.70	**<0.001**
Season [Late]		2.06	1.33-3.21	**0.001**
Random Effects				
σ^2		3.29		
$^{\tau}$00 Farm		0.00		
^{N} Farm	31			
Observations	357			
Marginal R^2 /Conditional R^2	0.037/NA			

Table 5.3 Number of bird fecal samples with arthropod natural enemy detections by arthropod family for our 5 focal bird species.

Species (n)	ANT	BRA	CAR	CHR	CLU	COC	DOL	EMP	HEM	PHI	RED	SAL	SYR	VES
American Goldfinch (61)	2	7	5	0	0	0	0	1	0	1	0	2	5	4
American Robin (53)	0	1	17	1	0	0	0	1	0	2	0	1	7	2
Savanah Sparrow (57)	1	11	10	1	1	2	0	0	1	0	0	0	7	3
Song Sparrow (83)	1	9	18	0	2	4	0	1	2	8	2	4	11	1
White-crowned Sparrow (103)	2	11	16	0	3	15	5	0	0	5	0	3	23	5
Total detections	6	39	66	2	6	21	5	3	3	15	2	10	53	15
	(1.68%)	(10.9%)	(18.5 %)	(0.56%)	(1.68%)	(5.88%)	(1.40%)	(0.84%)	(0.84%)	(4.20%)	(0.56%)	(2.80%)	(14.9%)	(4.2%)
Abbreviations: ANT: Anthocoridae. BRA = Braconidae. CAR = Carabidae. CHR = Chrysopidae. CLU = Cubionidae. COC = Coccinellidae. DOL = Dolichopodidae. EMP = Empididae. HEM = Hemerobiidae. PHI = Philodromidae. RED = Reduviidae. SAL = Salticidae. SYR = Syrphidae. VES = Vespidae.														

Table 5.4 Number of bird fecal samples with brassica pest family detections for our 5 focal bird species.

Bird Species (n)	Aphididae	Chrysomelidae	Noctuidae	Pieridae	Plutelldiae
American Goldfinch (61)	26	8	4	0	1
American Robin (53)	2	3	9	1	2
Savannah Sparrow (57)	14	8	8	0	5
Song Sparrow (83)	15	7	12	1	5
White-crowned Sparrow (103	23	15	24	0	3
Total detections (%)	80 (22.41%)	41 (11.5%)	57 (16%)	2 (0.56%)	16 (4.48%)

Chapter 6

Conclusion

The research presented in this dissertation highlights the complexity of gleaning causal connections in the web of myriad interactions that comprise an ecological system. This remains true even for the often "simplified" systems we see in agricultural settings. We attempted to better understand the role of generalist predators in organic agriculture, with a special focus on *P. melanarius* as an abundant and ubiquitous predator. An attempt to enhance molecular methods of examining the diet of *P. melanarius* on farms was not completely successful as the increased DNA concentration required for PCR-free library preparation (a prerequisite for shotgun metagenomics) proved to be a limiting factor. Still, we detected several potential brassica pests and, while there were also detections of predators, they were rare enough to make direct predation a minor consideration for *P. melanrius'* role as a disruptive force in biological control. While not conclusive, it serves as a solid proof of concept, and the method could be used to great effect with more refinement.

From there we extended our inquires to a higher level in Chapter 4, from a single organism to a larger community. Here we found that local factors were the predominant force in determining insect communities but the factors controlling brassica pests and their natural enemies were different. While once again there wasn't a direct benefit to crops, there is evidence that growers can make local changes that benefit predatory arthropods without also directly benefitting crop pests. Further research in this regard could therefore focus on refining the amount of habitat alterations needed and more specifically measuring the link with crop outcomes. While such community level analysis is useful, it is also a simplification and in Chapter 5 we once again expanded the scope on inquiry, this time looking at a higher trophic level, birds in this case, as well as even broader landscape factors. Once again while we failed to find evidence for significant crop benefits, there were important implications which would benefit from further study, such as the different probabilities for certain bird species having brassica pests or natural enemies in their diet. Combined with clear evidence that landscapes dictate the species comprising the bird community, it logically follows that some landscapes would better accommodate biological control over others.

As with the insect community study, the findings did not show large impacts but lay the groundwork for further inquiries. Like the molecular analysis, further methodology could be added to gain more actionable information. Stable isotope analysis (SIA) could be used to measure the relative importance of different trophic levels (herbivores vs predators or parasitoids) to the overall diet of individual birds. In addition, crop systems in which herbivory is more directly associated with lower yields might be more appropriate. Such an example would be kale where the sellable portion of the plant is eaten by pests as opposed to broccoli in which the major pests feed on the leaves while the head is what is sold. In the latter case only complete defoliation would cause a loss in production, and therefore biological control would only be beneficial in rare cases of extreme pest pressure.

Ultimately, the work presented here highlights both the benefits and challenges at a systems level approach when evaluating biological control systems. New technologies are making deeper molecular analysis possible, but methods need to be refined. The varying responses of pest taxa, arthropod natural enemies and bird communities also highlights the continued need for studies that account for multiple “real world” situations and reveals the limitations of tightly controlled experiments in predicting impacts in active farming systems. The work here provides a basis for more comprehensive studies which can help mold the trends found in this dissertation into more actionable recommendations to growers.

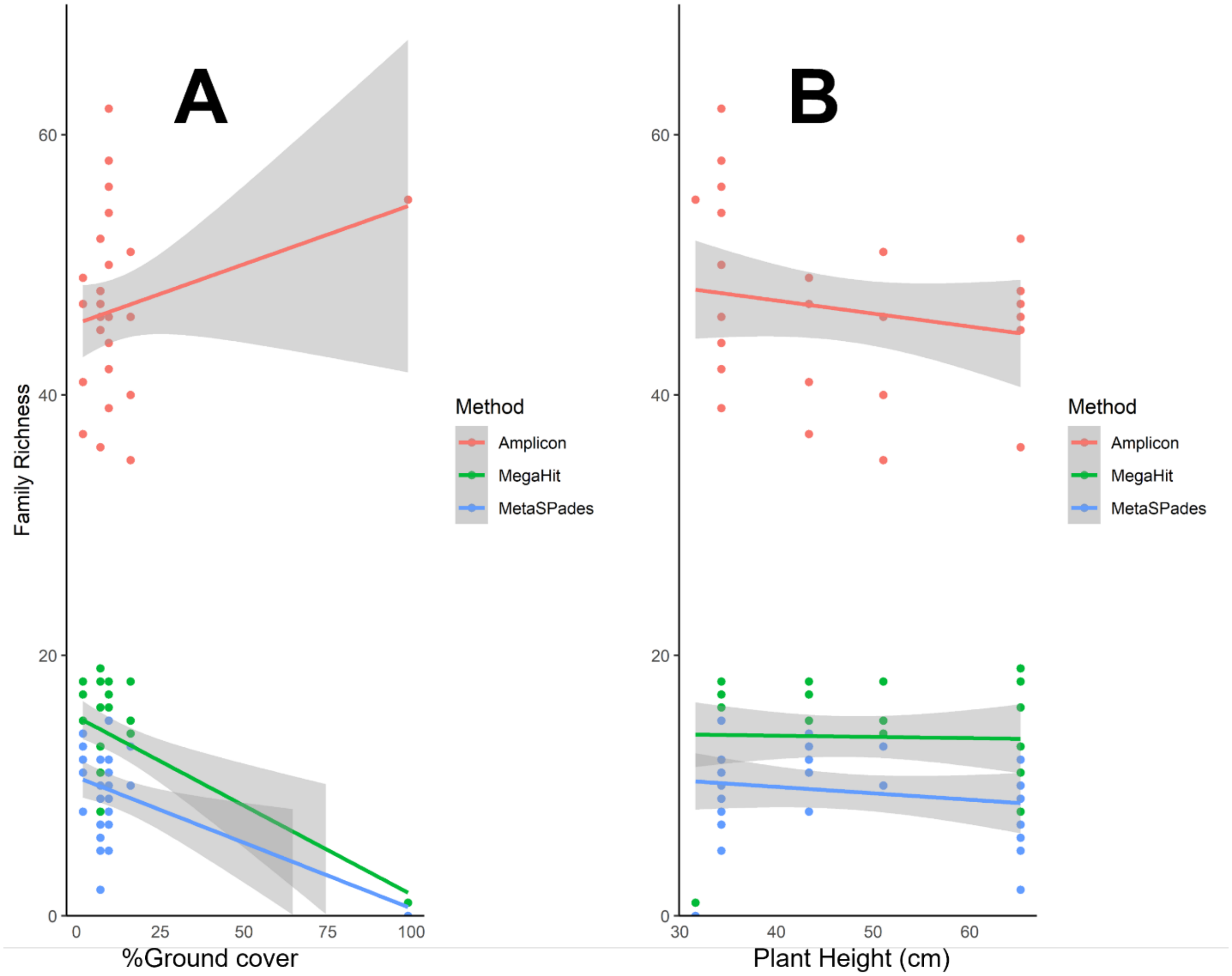
Appendices

Chapter 3 Appendix

NCBI blast 2.10.1+ parameters: -outfmt 6 -num_threads 20 -max_target_seqs 3 -max_hsps 1 -evalue 1e-6

Megan6 community edition parameters: Extended Mode; Min score: 500 bitscore; Max expected: 0.01; Min Percent identity: 90; Top percent 10; Min support Percent: 0.05; Min support: 10; Min Read Length: 500; LCA Algorithm: longReads; Percent to cover: 51.0; Read assignment mode: alignedBases.

Appendix Figure 1. Relationship between family richness and % ground cover (A) and plant height (B).



Appendix Table 1. Model sets used to test for factors influencing Family and Genera richness in *P. melanarius* diets.

Model Number	**Fixed Effects**	**Random Effects**
1	% cover	Farm
2	Method	Farm
3	Plant Height	Farm
4	% cover + Plant Height	Farm
5	% cover + Method	Farm
6	% cover + Method + Plant Height	Farm
7	Method + Plant Height	Farm
8		Farm

Chapter 4 Appendix

Appendix Table 2. Candidate model set considered for insect community analysis. The same candidate model set was considered for individual taxa abundances or activity-densities. P.seminatural = % seminatural cover (1000 m), Livestock = presence/absence of livestock on farm, X_NAT = % natural habit on farm, Round = early or late round of collection, Plant Height = average height of crops (cm). Ave. Weed cover = % of non-crop ground cover.

Model Number	**Fixed Effects**	**Random Effects**
1	p.seminatural	Farm
2	p.seminatural + Livestock	Farm
3	p.seminatural + Livestock + % NAT	Farm
4	p.seminatural + Livestock + % NAT + Round	Farm
5	p.seminatural + Livestock + % NAT + Round + Plant Height	Farm
6	p.seminatural + Livestock + % NAT + Round + Plant Height + Ave. weed cover	Farm
7	Livestock	Farm
8	Livestock + % NAT	Farm
9	Livestock + % NAT + Round	Farm
10	Livestock + % NAT + Round + Plant Height	Farm
11	Livestock + % NAT + Round + Plant Height + Ave weed cover	Farm
12	% NAT	Farm
13	% NAT + Round	Farm
14	% NAT + Round + Plant Height	Farm
15	% NAT + Round + Plant Height + Ave weed cover	Farm
16	Round	Farm
17	Round + Plant Height	Farm
18	Round + Plant Height + Ave weed cover	Farm
19	Plant Height	Farm
20	Plant Height + Ave weed cover	Farm
21	Ave weed cover	Farm
22		Farm

Appendix Table 3. Model selection results from insect community analysis. Only models within 2 **ΔAIC_c** of top model are displayed. Bold indicates effect sizes which were significant factors. Light grey items indicate effect sizes that were approaching significance ($p < 0.10$).

Dependent Variable	P.seminatural	Livestock	% NAT	Round	Plant.Height	Weed cover	ΔAIC_c	df	Weight
Ave.Aphids				1.146102	0.005164		0	5	0.2577
Ave.aphids				1.071058	0.004920	0.012132	0.9	6	0.1673
Ave.aphids		-0.861795	-0.572043	1.411052	0.004241		1.8	7	0.1053
Ave.aphids			-1.697359	1.15898	0.005047		2.1	6	0.0878
P. xyllostella abundance				0.880717	0.004619		0	5	0.2485
P. xyllostella abundance					0.05619		0.5	4	0.1893
P. xyllostella abundance				0.855046	0.004482	0.009312	1.7	6	0.1076
P. xyllostella abundance					0.05443	0.01124	1.9	5	0.0982
P. xyllostella abundance		0.999615	-3.057311	0.671782	0.005192		1.9	7	0.0938
P.rapae abundance							0	3	0.1809
P.rapae abundance			-11.796				0.5	4	0.1381

P.rapae abundance						0.02266	0.9	4	0.1181
Coccinellidae abundance						0.2018	0	4	0.1753
Coccinellidae abundance	-2.1497						1.1	4	0.1027
Coccinellidae abundance					0.01972	0.01916	1.4	5	0.0854
Coccinellidae abundance				0.9199			1.5	4	0.0824
Coccinellidae abundance							1.6	3	0.077
Chrysoperla abundance					-0.01310	0.08396	0	4	0.3225
Chrysoperla abundance		-2.990e+01					0.7	5	0.2280
Nabis abundance	-2.3305						0	4	0.1435
Nabis abundance	-2.4520	0.7700					0.4	5	0.1153
Carabidae activity density						0.020358	0	4	0.4582
Total Richness						0.008802	0	4	0.3929
Total richness					0.007036	0.008287	0.8	5	0.2636

Foliar Predator Richness						0.013012	0	4	0.2718
Foliar Predator Richness						0.014451	0.1	5	0.2582
Foliar Predator Richness				0.442195	-0.002294	0.014052	0.6	6	0.2037
Total Abundance						0.012898	0	4	0.2953
Total Abundance					0.009553	0.012111	1.5	5	0.1391

Appendix Table 4. Model results for pest taxa abundance as predicted by Foliar predator richness. No significant associations. Models had Farm as a random effect.

Pest	Estimate	SE	P value
P. crucifierae	-0.2194	0.5844	0.7073
Aphids	0.01581	0.23302	0.946
P. xyllostella	-0.11114	0.21881	0.612
P. rapae	0.5366	0.3469	0.1219

Chapter 5 Appendix

Appendix Table 5. Model selection results predicting aphid abundance per plot by treatment (bird access/exclusion), survey occasion (mid-growth/late growth), and % natural cover (1 km). Models having > 5% of model weights (ω) are shown. Bolded values indicate $P < 0.05$ (only indicated for models within 2 ΔAIC_c of the top model). Numbers in columns treatment through % natural are model estimate (standard error). Cage nested within farm is included as a random effect. Bird exclusion and survey 1 (mid-growth) are reference groups for treatment and survey, respectively. Next-best model not shown had $\Delta AIC_c = 4.3$ and $\omega = 0.037$.

Model	**Treatment (bird access)**	**Survey (late growth)**	**Treatment (bird access * Survey)**	**% Natural**	**ΔAIC_c**	**df**	**Weight**
Treatment + survey	**0.92 (0.43)**	**2.59 (0.17)**			0	5	0.32
Treatment * survey	**1.62 (0.67)**	**2.83 (0.25)**	-0.44 (0.33)		0.4	6	0.26
Treatment + survey + % natural	0.92 (0.43)	2.59 (0.17)		-0.0007 (0.0083)	2.2	6	0.11
Survey		2.58 (0.17)			2.5	4	0.093
Treatment * survey + % natural	1.17 (0.47)	2.83 (0.25)	-0.44 (0.33)	-0.0007 (0.0084)	2.6	7	0.088

Appendix Table 6. Model selection results predicting caterpillar abundance per plot by treatment (bird access/exclusion), survey occasion (mid-growth/late growth), and % natural cover (1 km). Models having > 5% of model weights (ω) are shown. Bolded values indicate $P < 0.05$ (only indicated for models within 2 ΔAIC_c of the top model). Numbers in columns treatment through % natural are model estimate (standard error). Cage nested within farm is included as a random effect. Bird exclusion and survey 1 (mid-growth) are reference groups for treatment and survey, respectively. Next-best model not shown had $\Delta AIC_c = 4.9$ and $\omega = 0.029$.

Model	**Treatment (bird access)**	**Survey (late growth)**	**% Natural**	**ΔAIC_c**	**df**	**Weight**
Null				0	3	0.33
% Natural			0.0035 (0.0040)	1.3	4	0.17
Treatment	-0.15 (0.20)			1.5	4	0.15
Survey		-0.056 (0.14)		1.9	4	0.12
Survey + % Natural		-0.055 (0.14)	0.0035 (0.0040)	3.3	5	0.063
Treatment + Survey	-0.15 (0.20)	-0.056 (0.14)		3.5	5	0.057

Appendix Table 7. Model selection results predicting flea beetle abundance per plot by treatment (bird access/exclusion), survey occasion (mid-growth/late growth), and % natural cover (1 km). Models having > 5% of model weights (ω) are shown. Bolded values indicate $P < 0.05$ (only indicated for models within 2 ΔAIC_c of the top model). Numbers in columns treatment through Treatment * survey * % natural are model estimate (standard error). Cage nested within farm is included as a random effect. Bird exclusion and survey 1 (mid-growth) are the reference groups for treatment and survey, respectively. Next-best model not shown had $\Delta AIC_c = 23.0$ and $\omega < 0.001$.

Model	**Treatment (bird access)**	**Survey (late growth)**	**Treatment (bird access) * Survey (late growth)**	**% Natural**	**Survey (late growth) * % Natural**	**Treatment (bird access) * % Natural**	**Treatment (bird access) * Survey (late growth) * % Natural**	**ΔAIC_c**	**df**	**Weight**
Survey * % Natural		**-5.41 (0.74)**		**-0.13 (0.024)**	**0.11 (0.021)**			0	6	0.70
Treatment + Survey * % Natural	0.089 (0.79)	-5.41 (0.74)		-0.13 (0.024)	0.11 (0.021)			2.2	7	0.23
Treatment * Survey * % Natural	1.03 (1.61)	-5.27 (0.96)	-0.59 (1.43)	-0.12 (0.029)	0.095 (0.025)	-0.049 (0.044)	0.049 (0.040)	4.6	10	0.071

Appendix Table 8. Model selection results predicting aphid mummy abundance per plot by treatment (bird access/exclusion), survey occasion (mid-growth/late growth), and % natural cover (1 km). Models having > 5% of model weights (ω) are shown. Bolded values indicate $P < 0.05$ (only indicated for models within 2 ΔAIC_c of the top model). Numbers in columns treatment through survey * %

natural are model estimate (standard error). Cage nested within farm is included as a random effect. Bird exclusion and survey 1 (mid-growth) are the reference groups for treatment and survey, respectively. Next-best model not shown had $\Delta AIC_c = 4.0$ and $\omega = 0.040$.

Model	**Treatment (bird access)**	**Survey (late growth)**	**Treatment (bird access) * Survey (late growth)**	**% Natural**	**Survey (late growth) * % Natural**	**ΔAIC_c**	**df**	**Weight**
Treatment + survey	**1.00 (0.48)**	**1.37 (0.20)**				0	5	0.30
Treatment * survey	**1.35 (0.56)**	**1.69 (0.31)**	-0.53 (0.40)			0.4	6	0.24
Treatment + survey + % natural	**1.00 (0.48)**	**1.37 (0.20)**		-0.0050 (0.0090)		1.9	6	0.12
Survey		1.36 (0.20)				2.3	4	0.094
Treatment * survey + % natural	1.35 (0.56)	1.69 (0.31)	-0.52 (0.40)	-0.0047 (0.0091)		2.3	7	0.092
Treatment + survey * % natural	1.00 (0.48)	1.57 (0.32)		-0.0020 (0.0097)	-0.0050 (0.0064)	3.5	7	0.053

Appendix Table 9. Model selection results predicting proportion parasitized vs. unparasitized aphids per plot by treatment (bird access/exclusion), survey occasion (mid-growth/late growth), and % natural cover (1 km). Models having > 5% of model weights (ω) are shown. Bolded values indicate $P < 0.05$ (only indicated for models within 2 ΔAIC_c of the top model). Numbers in columns treatment through survey * % natural are model estimate (standard error). Cage nested within farm is included as a random effect. Bird exclusion and survey 1 (mid-growth) are the reference groups for treatment and survey, respectively. Next-best model not shown had $\Delta AIC_c = 4.9$ and $\omega = 0.031$.

Model	**Treatment (bird access)**	**Survey (late growth)**	**Treatment (bird access) * Survey (late growth)**	**% Natural**	**Survey (late growth) * % Natural**	**ΔAIC$_c$**	**df**	**Weight**
Survey		**-0.96 (0.18)**				0	4	0.35
Treatment + Survey	0.22 (0.22)	**-0.95 (0.18)**				1.2	5	0.20
% Natural + Survey		**-0.95 (0.19)**		-0.0025 (0.0039)		1.7	5	0.15
Treatment + Survey + % Natural	0.21 (0.22)	-0.94 (0.19)		-0.0024 (0.0039)		2.9	6	0.082
Treatment * Survey	0.32 (0.32)	-0.84 (0.30)	-0.18 (0.38)			3.1	6	0.075
% Natural * Survey		-0.89 (0.30)		-0.0016 (0.0049)	-0.0017 (0.0062)	3.8	6	0.053

Appendix Table 10. Model selection results predicting predator abundance per plot by treatment (bird access/exclusion), survey occasion (mid-growth/late growth), and % natural cover (1 km). Models having > 5% of model weights (ω) are shown. Bolded values indicate $P < 0.05$ (only indicated for models within 2 ΔAIC_c of the top model). Numbers in columns treatment through survey * % natural are model estimate (standard error). Cage nested within farm is included as a random effect. Bird exclusion and survey 1 (mid-growth) are the reference groups for treatment and survey, respectively. Next-best model not shown had $\Delta AIC_c = 3.9$ and $\omega = 0.048$.

Model	**Treatment (bird access)**	**Survey (late growth)**	**Treatment (bird access) * Survey (late growth)**	**% Natural**	**Survey (late growth) * % Natural**	**ΔAIC_c**	**df**	**Weight**
Survey		**1.16 (0.23)**				0	4	0.34
Treatment * Survey	0.59 (0.40)	**1.55 (0.33)**	-0.73 (0.45)			1.4	6	0.17
Treatment + Survey	0.15 (0.29)	**1.17 (0.23)**				1.9	5	0.13
% Natural + Survey		1.16 (0.23)		-0.0020 (0.0055)		2.0	5	0.13
% Natural * Survey		0.86 (0.39)		-0.0064 (0.0073)	0.0073 (0.0079)	3.3	6	0.065
Treatment * Survey + % Natural	0.59 (0.40)	1.56 (0.33)	-0.74 (0.45)	-0.0026 (0.0056)		3.4	7	0.062

Appendix Table 11. Model selection results predicting spider abundance per plot by treatment (bird access/exclusion), survey occasion (mid-growth/late growth), and % natural cover (1 km). Models having > 5% of model weights (ω) are shown. Bolded values indicate $P < 0.05$ (only indicated for models within 2 ΔAIC_c of the top model). Numbers in columns treatment through survey * %

natural are model estimate (standard error). Cage nested within farm is included as a random effect. Bird exclusion and survey 1 (mid-growth) are the reference groups for treatment and survey, respectively. Next-best model not shown had $\Delta AIC_c = 4.1$ and $\omega = 0.042$.

Model	**Treatment (bird access)**	**Survey (late growth)**	**% Natural**	**Survey (late growth) * % Natural**	**ΔAIC_c**	**df**	**Weight**
Survey * % Natural		-0.25 (0.33)	**-0.018 (0.007)**	**0.017 (0.0080)**	0	6	0.33
Treatment + Survey * % Natural	0.16 (0.24)	-0.25 (0.33)	**-0.018 (0.007)**	**0.017 (0.0080)**	1.7	7	0.14
Survey + % Natural		0.33 (0.20)	-0.0075 (0.0049)		2.5	5	0.093
Survey		0.35 (0.20)			2.7	4	0.084
% Natural			-0.0080 (0.0049)		3.0	4	0.074
Null					3.5	3	0.056

Appendix Table 12. Model selection results predicting syrphid larvae abundance per plot by treatment (bird access/exclusion), survey occasion (mid-growth/late growth), and % natural cover (1 km). Models having > 5% of model weights (ω) are shown. Bolded values indicate P < 0.05 (only indicated for models within 2 ΔAIC_c of the top model). Numbers in columns treatment through survey * treatment are model estimate (standard error). Cage nested within farm is included as a random effect. Bird exclusion and survey 1 (mid-growth) are the reference groups for treatment and survey, respectively. Next-best model not shown had $\Delta AIC_c = 4.9$ and ω = 0.021.

Model	**Treatment (bird access)**	**Survey (late growth)**	**% Natural**	**Survey (late growth) * % Natural**	**Treatment (bird access) * Survey (late growth)**	**ΔAIC_c**	**df**	**Weight**
Survey		**1.80 (0.43)**				0	4	0.24
Survey * % Natural		**2.87 (0.90)**	**0.028 (0.014)**	-0.021 (0.014)		0.4	6	0.20
Survey + % Natural		**1.80 (0.43)**	0.012 (0.0092)			0.6	5	0.18
Treatment + Survey	0.12 (0.49)	1.80 (0.43)				2.1	5	0.086
Treatment * Survey	1.10 (0.92)	2.51 (0.76)			-1.19 (0.92)	2.4	6	0.074
Treatment + Survey * % Natural	0.13 (0.49)	2.87 (0.90)	0.028 (0.014)	-0.021 (0.014)		2.5	7	0.068
Treatment + Survey + % Natural	0.11 (0.49)	1.80 (0.43)	0.012 (0.009)			2.7	6	0.063
Treatment * Survey + % Natural	1.12 (0.92)	2.51 (0.76)	0.012 (0.009)		-1.22 (0.91)	3.0	7	0.056

Appendix Table 13. Model selection results predicting average proportion leaf damage per plot by treatment (bird access/exclusion), survey occasion (mid-growth/late growth), and % natural cover (1 km). Models having > 5% of model weights (ω) are shown. Bolded values indicate $P < 0.05$ (only indicated for models within 2 ΔAIC_c of the top model). Numbers in columns treatment through survey * treatment are model estimate (standard error). Cage nested within farm is included as a random effect. Bird exclusion and survey 1 (mid-growth) are the reference groups for treatment and survey, respectively. Next-best model not shown had $\Delta AIC_c = 5.4$ and ω = 0.016.

Model	**Treatment (bird access)**	**% Natural**	**Survey (late growth)**	**Survey (late growth) * % Natural**	**Treatment (bird access) * % Natural**	**Treatment (bird access) * Survey (late growth)**	**ΔAIC_c**	**df**	**Weight**
% Natural * Survey		**-0.021 (0.006)**	**-0.73 (0.27)**	0.0094 (0.0062)			0	6	0.22
% Natural + Survey		**-0.016 (0.0043)**	**-0.39 (0.15)**				0.1	5	0.22
% Natural + Survey + Treatment	0.28 (0.21)	**-0.016 (0.0043)**	**-0.39 (0.15)**				0.4	6	0.19
% Natural * Survey + Treatment	0.27 (0.21)	**-0.021 (0.006)**	**-0.72 (0.27)**	0.0091 (0.0062)			0.5	7	0.18
% Natural * Treatment + Survey	0.065 (0.38)	-0.019 (0.0062)	-0.39 (0.15)		0.0057 (0.0084)		2.1	7	0.078

% Natural + Treatment * Survey	0.28 (0.25)	-0.016 (0.0043)	-0.39 (0.22)			0.0011 (0.30)	2.6	7	0.062

Appendix Table 14. Model selection results predicting detection of pest taxa (first half of table) and arthropod natural enemy taxa (second half of table) in bird feces by bird species, survey occasion (early vs. late season captures), and % natural cover (1 km). Models having > 5% of model weights (ω) are shown. Numbers in columns species through season * % natural indicate results of likelihood ratio tests on model terms. Farm is included as a random effect. Pests: next-best model not shown had $\Delta AIC_c = 2.7$ and ω = 0.047. Natural enemies: next-best model not shown had $\Delta AIC_c = 3.7$ and ω = 0.038.

Prey detected (response variable)	**Model (predictor variables)**	**Bird species**		**Season**		**% Natural**		**Season* % Natural**		**ΔAIC_c**	**df**	**Weight**
		LRT χ^2	**P - value**	**LRT χ^2**	**P-value**	**LRT χ^2**	**P-value**	**LRT χ^2**	**P-value**			
Pest	Season			**10.60**	**0.0011**					0	3	0.18
Pest	% Natural + Season			**9.84**	**0.0017**	1.77	0.18			0.3	4	0.16
Pest	% Natural * Season							1.95	0.16	0.4	5	0.15
Pest	Species + Season * % Natural	8.01	0.091					2.96	0.085	0.7	9	0.12
Pest	Species + Season	6.84	0.14	3.73	0.055					1.4	7	0.088
Pest	Species + Season + % Natural	7.00	0.14	3.19	0.074	1.94	0.16			1.6	8	0.081
Enemy	Species + Season	**9.52**	**0.049**	3.08	0.079					0.0	7	0.25

Enemy	Species	**10.95**	**0.027**							1	6	0.15
Enemy	Season			4.51	0.034					1.3	3	0.13
Enemy	Species + Season + % Natural	**9.70**	**0.046**	2.90	0.089	0.49	0.49			1.6	8	0.11
Enemy	Species + % Natural	**11.17**	**0.025**			0.66	0.42			2.4	7	0.074
Enemy	Species + Season * % Natural	**9.78**	**0.044**					4.38	0.22	2.9	9	0.058
Enemy	Season + % Natural			**4.37**	**0.037**	0.31	0.57			3.0	4	0.055

Appendix Table 15. Tukey HSD test comparing detection of pest insects by bird species from the best-supported model including fixed effects of species and survey period, with farm included as a random effect. Bird species names given in 4-letter AOU codes.

	AMGO		**AMRO**		**SAVS**		**SOSP**	
	Estimate (SE)	**P -value**	**Estimate (SE)**	**P-value**	**Estimate (SE)**	**P-value**	**Estimate (SE)**	**P-value**
AMGO			0.51 (0.42)	0.74				
AMRO								
SAVS	0.66 (0.40)	0.45	0.15 (0.42)	0.99				
SOSP	0.73 (0.37)	0.29	0.22 (0.36)	0.97	0.070 (0.37)	0.99		
WCSP	**1.04 (0.35)**	**0.027**	0.54 (0.36)	0.56	0.31 (0.31)	0.81	0.31 (0.31)	0.84

Appendix Table 16. Number of brassica pest species detections in bird feces for our five focal bird species.

Bird Species	***Phyllotreta striolata***	***Plutella xylostella***	***Pieris rapae***	***Trichoplusia ni***	***Brevicoryne brassicae***	***Myzus persicae***
American Goldfinch	0	1	0	0	2	1
American Robin	0	2	0	0	0	1
Savannah Sparrow	1	5	0	0	0	1
Song Sparrow	3	5	1	0	0	7
White-crowned Sparrow	1	3	0	1	0	5
Total detections (%)	**5 (1.40%)**	**16 (4.48%)**	**1 (0.28%)**	**1 (0.28%)**	**2 (0.56%)**	**15 (4.20%)**