Avian Ecosystem Services and Disservices Within a Mixed Landscape of Intensive Agriculture and Grassland

BY

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THESIS

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SUMMARY

Much of the Midwest region of the United States is farmed intensively for corn and soybean production. Grasslands and prairies, once abundant in this landscape, are now often found in small patches embedded within an agricultural matrix. These grasslands and prairies provide habitat for source populations of birds, which can forage both in natural and nearby cultivated habitats. Birds can provide ecosystem services in this system by consuming herbivorous pest arthropods, or disservices by consuming beneficial predatory arthropods that would otherwise consume pests. I conducted a series of experimental and observational studies to examine the pest control services and disservices provided by birds within this study system.

In chapter one, I conducted an exclosure experiment where I used cages to exclude birds from corn and soybean crops at a site with an adjacent prairie. I also conducted a DNA barcoding diet study to determine what species of arthropods the birds most commonly consumed. I found that birds provided significant net services in corn crops, but significant net disserves in the adjacent soybean crops. I estimated that birds in this system provided a service worth approximately US \$275/ha in corn yield gain, and a disservice valued at approximately \$348/ha in soybean yield loss. The diet analysis revealed a possible explanation for the fact that birds provided services in corn and disservices in soybeans: I found that many bird fecal samples contained DNA from both beneficial predatory arthropods and known economically-significant pests of corn, but few economically-significant pests of soybeans.

In chapter two, I further examined the disservices that birds provide in soybean crops. I again conducted exclosure experiments in soybeans, but added additional study sites and measured the effect of birds on both leaf damage by pests and grain yield. I found that exclosure plots had lower levels of leaf damage by pests than control plots, but there was no resulting

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effect on crop yield. I also found that sites with higher bird species richness had overall lower levels of leaf damage by pests. These results suggest that although birds may have released pests through intraguild predation, there was no net disservice when considering crop yield during this study season.

In chapter three I further examined the diets of grassland/soybean field bird communities. I again conducted a DNA barcoding diet analysis, this time at multiple sites. I compared the diets of different bird species and birds captured at different sites. I found that the bird community consumed an extremely wide variety of prey species, and that diets differed by both bird species and capture site. My findings suggest that birds responded opportunistically to prey availability at each site, and are likely able to respond quickly to pest outbreaks. I also found that birds generally consumed more herbivorous arthropods than natural enemy, or "beneficial," arthropods.

The combined results of this dissertation suggest that birds have the potential to provide surprisingly large economic effects within an intensively farmed corn and soybean landscape. Grassland and prairie habitat near farmland likely provides a source population of birds that provide these top-down trophic interactions. Furthermore, birds likely provide services beyond those provided by beneficial arthropod natural enemies. These findings suggest there are both economic and ecological reasons that future study should continue to examine bird effects in corn and soybean agriculture.

1. INTRODUCTION

1.1. Why Ecosystem Services?

The idea that nature provides services to humans is ancient, although it has been formalized much more recently in the scientific vernacular (Costanza et al. 2017). Ecosystem services are defined as "the benefits people obtain from ecosystems," including benefits derived both directly and indirectly (MEA 2005). When we define services, we must also define disservices; ecosystem disservices are negative effects caused by ecosystems. Again, they have long been considered, but more recently defined (Shapiro and Báldi 2014). When we can quantitatively show that ecosystem services outweigh the disservices, we can give an economic reason to conserve the natural capital that produces those services.

When I was first introduced to the concept of ecosystem service valuation, I appreciated the importance of economically incentivizing conservation: it allows conservationists to speak the same language as decision-makers. Some ecologists argue that this approach might not be the most effective at promoting biodiversity, because it ignores the intrinsic value inherent in nature (Bekessy et al. 2018). This may be true when we consider ways to sway general public opinion, but the concept of ecosystem services doesn't have to be a universal approach to decision-making. When we consider large industries like agriculture, framing conservation in terms of quantified ecosystem services makes sense. Agriculture is an extremely valuable industry worldwide, on top of the importance it holds in maintaining food security for almost every society. United States farming output, alone, contributed \$132.8 billion USD to the US gross domestic product (GDP) in 2017 (USDA ERS 2020). When people and governments make decisions about agriculture, it makes sense that they need to take economics into account.

Conservationists must consider biodiversity in the context of agriculture because agriculture is responsible for so much biodiversity loss at a global scale. Approximately 11 percent of the global land area is currently used to produce crops (Bruinsma 2003). Agriculture negatively affects biodiversity in numerous ways: by converting natural land for growing crops, increasing erosion and introducing agricultural runoff into bodies of water, by contributing to climate change, and so on. For these reasons, I was drawn to an ecosystem-services approach to studying the ways we can reconcile biodiversity with agricultural production.

One important ecosystem service to consider in agriculture is pest control. The potential loss of crops worldwide due to animal pests is approximately 18%, although this estimate varies widely depending on region and crop type (Oerke 2006). Birds, which have been estimated to consume approximately 400-500 million tons of arthropod prey per year (Nyffeler et al. 2018), certainly provide valuable arthropod pest control services in agriculture. As someone who was interested in learning about both birds and conservation, I found this to be an appealing area of study.

I began studying pest control services provided by birds on farms while completing my Master's degree. The small organic farms where I conducted my Master's research were wild and beautiful. I counted over 100 species of birds at my study sites, and I could see rivers and redwood trees from the edges of the farm fields (Garfinkel and Johnson 2015). Many of the farmers were already in touch with the ecosystems around them, and sought to encourage the birds and other wildlife that could be helpful to them. I had loved working in this agricultural system, but I knew that the small, organic farms I had studied represented a miniscule proportion of the farmed land in the United States.

The giant corn and soybean farms in the Midwest region of the United States are more representative of much of the land farmed in this country. In states like Illinois, corn and soybean agriculture is by far the main land use (Figure 1). Meanwhile, less than one percent of the original tallgrass prairie remains in the Midwest (Ricketts 1999). I felt that studying birdprovisioned services with a corn-soybean-prairie system had the potential for impact on a larger scale than my previous research.

1.2. Beyond Ecosystem Services

This dissertation is a report of the successful studies I conducted to examine avian services and disservices in the Midwest. It does not include my failed attempts at experiments, the projects I was unable to complete due to time and logistical constraints, and the projects I imagined I would do with unlimited funding and time. I have focused in these writings on the applied aspects of ecology: the economic effect of birds, the value of grasslands within a largely agricultural matrix. But there is so much more to learn here, from determining the ecosystem properties that affect the differing strengths of trophic cascades, to examining selective vs. opportunistic foraging strategies, to the natural history of goldfinch consumption of insects. I hope that this dissertation serves to convince others that this study system is more interesting from an ecological perspective than one could possibly believe when first looking out on the vast seas of corn and soybeans in the Midwest.



Figure 1. Corn and Soybean fields in Illinois, USA, in 2016. Data from USDA Cropland Data Layer (CDL), National Agricultural Statistics Service, 2016.

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2. BIRDS SUPPRESS PESTS IN CORN BUT RELEASE THEM IN SOYBEAN CROPS WITHIN A MIXED PRAIRIE/AGRICULTURE SYSTEM

This chapter was previously published as: Garfinkel, M. B., E. S. Minor, and C. J. Whelan (2020). Birds suppress pests in corn but release them in soybean crops within a mixed prairie/agriculture system. The Condor:duaa009.

2.1. ABSTRACT

Birds provide ecosystem services (pest control) in many agroecosystems and have neutral or negative ecological effects (disservices) in others. Large-scale, conventional row crop agriculture is extremely widespread globally, yet few studies of bird effects take place in these agroecosystems. We studied indirect effects of insectivorous birds on corn and soybean crops in fields adjacent to a prairie in Illinois (USA). We hypothesized that prairie birds would forage for arthropods in adjacent crop fields and that the magnitude of services or disservices would decrease with distance from the prairie. We used bird-excluding cages over crops to examine the net effect of birds on corn and soybean grain yield. We also conducted DNA metabarcoding to identify arthropod prey in fecal samples from captured birds. Our exclosure experiments revealed that birds provided net services in corn and net disservices in soybeans. Distance from prairie was not a significant predictor of exclosure treatment effect in either crop. Many bird fecal samples contained DNA from both beneficial arthropods and known economicallysignificant pests of corn, but few economically-significant pests of soybeans. Song Sparrows (Melospiza melodia), one of our most captured species, most commonly consumed corn rootworms, an economically-significant pest of corn crops. We estimated that birds in this system provided a service worth approximately US \$275/ha in corn yield gain, and a disservice valued at approximately \$348/ha in soybean yield loss. Our study is the first to demonstrate that birds can provide substantial and economically valuable services in field corn, and disservices in soybean crops. The contrasting findings in the two crop systems suggest a range of bird impacts

within widespread agro-ecosystems and demonstrate the importance of quantifying net trophic effects.

2.2 INTRODUCTION

Trophic cascades are well-studied in agroecosystems because of their potential to produce economically valuable biological control. Agroecosystems often provide unusually strong trophic cascades for terrestrial systems owing to their simple food webs and high productivity (Halaj and Wise, 2001). However, the majority of studies of trophic cascades and food web interactions in agricultural systems focus on arthropods as both pests and predators (e.g. Chaplin-Kramer et al. 2011, Liere et al. 2015) and ignore vertebrate taxa. Vertebrate taxa, however, including insectivorous and omnivorous birds, may drive substantial positive or negative effects on crops with resulting economic impacts (Sekercioglu et al., 2016). Because most insectivorous and omnivorous bird species are generalist arthropod predators (i.e. they may consume both predatory and herbivorous arthropods), they may provide a combination of both services and disservices simultaneously. Therefore, it is important to know the net effects birds provide under different agricultural conditions (Peisley et al. 2015, Pejchar et al. 2018).

A number of studies have shown that birds may provide substantial pest control services in a variety of agricultural systems through top-down control of pests (Whelan et al. 2008, Sekercioglu et al. 2016). Others have shown neutral effects (e.g. Garfinkel and Johnson 2015), direct negative effects caused by damaging or consuming crops (Gebhardt et al., 2011; Hannay et al., 2019; Lindell and Eaton, 2012), or indirect negative effects when birds release pests through intraguild predation of predatory arthropods (e.g. Grass et al. 2017, Tschumi et al. 2018). Some of these studies show bird-provisioned services or disservices in terms of plant damage or pest density, without reporting resulting effects on crop yields (e.g. Van Bael et al. 2007, Koh 2008).

However, plants can tolerate a certain amount of damage before they reach an economic injury level that reduces crop yield (Pedigo et al., 1986). Therefore, bird-provisioned services and disservices in agricultural systems are most meaningful when put in the context of crop yield instead of only plant damage or pest density (Whelan et al., 2008). These data can then be used to extrapolate approximate economic gains or losses due to bird services or disservices (e.g. Kellermann et al. 2008).

Few studies on bird trophic effects have taken place in large-scale conventional row crop agriculture (but see Kross *et al.* 2016). Instead, they have mainly been conducted on small scale farms or agroforestry systems, often in the tropics, that tend to harbor larger or more diverse bird communities (Sekercioglu et al. 2016). In the United States, corn and soybeans are by far the most widely grown row crops, with a total combined area of over 69.7 million ha harvested in 2017 (USDA: NASS 2018). Considering the large extent of corn and soybean agriculture, it is surprising that there is little information on the effects of birds in these systems.

While corn and soybean fields are not known for their bird abundance or diversity, fields in the Midwest region of the United States (where corn and soybean crop cultivation is concentrated) are sometimes adjacent to remnant or restored prairies and grasslands. These "natural" or uncultivated habitats may provide sources of birds that also forage within the agricultural fields (Rodenhouse and Best 1983). If prairie birds provide significant indirect effects on crops in large-scale conventional row crop agriculture, these effects may be stronger in crops close to these remnant and restored prairies. Such distance effects have been documented in other agricultural systems, including coffee (Karp et al., 2013), cacao (Maas et al., 2015), and tropical forest plantations (Roels et al., 2018).

We used two approaches to examine the indirect effects of bird predation on crop pests in corn and soybean fields adjacent to prairie. First, we used bird-excluding cages ("exclosures") over crop plants to determine whether birds provide net pest control services or disservices. If the prairie provides source habitat for birds, we hypothesized that the strength of these services or disservices would decline with distance from the prairie. Furthermore, crop field edges often host higher densities of some pest species (Nguyen and Nansen, 2018), and we expected these greater prey densities would exacerbate differences between the exclosure and control treatments near the field edges. Second, we used a DNA metabarcoding diet analysis to determine whether birds captured in corn and soybean fields and adjacent prairie consume known crop pests or beneficial predatory arthropods. If birds collectively provide net services in the corn and soybean fields, we would expect to find evidence that birds predominantly consume economically-significant pests. If they collectively provide net disservices, we would expect that the birds predominantly consume beneficial arthropods.

2.3 METHODS

2.3.1 <u>Study Site</u>

We conducted this study at Nachusa Grasslands, a system of restored and remnant tallgrass prairie in northern Illinois, USA (41°54' N, 89°19' W). Nachusa Grasslands, which is owned by the Nature Conservancy (TNC), is embedded within a landscape dominated by corn and soybean agriculture. The property is approximately 1,538 ha and includes some agricultural fields that are leased to farmers until TNC is ready to restore them to prairie.

We conducted experiments in two agricultural fields owned by Nachusa Grasslands: one planted in corn (*Zea mays*, \sim 21 ha) and another planted in soybeans (*Glycine max*, \sim 17 ha). The fields were leased to two different farmers (one to farm each crop), and were managed in the

same ways as other fields in the region. The two crops were not separated by any cleared margin, and both crops shared an edge with a mature restored tallgrass prairie fragment (Figure 2). The corn variety grown in our study system was genetically modified to express Bt (*Bacillus thuringiensis*) toxins, and the soybeans were sprayed on July 26 with Hero® (FMC Global Specialty Solutions), a broad-spectrum insecticide. The farmer raised the boom arm of the tractor over the exclosures while spraying, so the exclosures were not removed for this. Although there were some scattered shrubs along the southern border of the soybean field, and the western border of the corn and soybean fields, there were no hedgerows or treelines separating the cropland from the prairie, or within 500 m of the experimental set up.



Figure 2. Study site in northern Illinois, USA. Exclosure and control plots within a pair are separated by 2 m, and exclosure representations are not sized to scale. The land to the south of our study site is grassland, and all exclosures are greater than 50 m from the southern border of the soybean field.

2.3.2 Exclosures

During the growing season of 2016, we placed six bird exclosures in the corn field, and six in the soybean field. Each exclosure was paired with a control plot marked with small plastic plant tags and located 2 m from the exclosure. We placed half of the exclosures and control plots 5 m from the prairie/crop edge, and the other half 55 m into the field interior to test for an effect of distance from the prairie edge. We expected this distance to be outside the foraging range of most birds living in the prairie, as previous research using experimental feeders placed in other crop fields showed that most bird species foraged within 20 m of the field edge (Puckett et al., 2009).

The exclosure frames were constructed from PVC pipe covered with clear nylon monofilament netting (1.9 cm square, 3.8 cm stretch mesh). The mesh size was small enough to exclude even the smallest birds found at the site (e.g. Common Yellowthroats [*Geothlypis trichas*] and Field Sparrows [*Spizella pusilla*]) but allow access to larger arthropods such as grasshoppers (Orthoptera) and butterflies (Lepidoptera). Costamagna *et al.* (2008) found that exclosures using much finer mesh than ours had no direct effect on soybean grain yield, so we expected that any treatment effect would be due to bird exclusion and not changes in crop microclimate. The exclosure footprint was 1.5 m x 0.6 m. The exclosures placed over soybeans were 1.5 m tall and those over corn were 3 m tall to accommodate plant growth. Each exclosure covered different numbers of plants (5–10) depending on planting density and row width. To control for this, as well as to avoid measuring the plants against the side of the exclosure that could potentially push leaves out through the netting, we focused on the central five plants in the exclosure and marked them with small plastic plant tags. We placed the exclosures over the crops once they had clearly sprouted in the field and were at least 5 cm tall (mid-June 2016). We

removed them approximately one week before the farmers harvested the fields (early October 2016) for a total exclusion period of approximately 3.5 months.

Upon removal of the exclosures, we hand-harvested the crops from the five marked central plants inside the exclosure, and the five marked plants from each control plot. We removed the corn kernels from the cobs and the soybeans from the pods, oven dried them to remove all water weight, and recorded the total dry biomass of crop yield per plant (hereafter referred to as grain yield).

2.3.3 Collection of Fecal Samples

We operated mist nets twice in June, twice in July, and three times in August 2016 to capture birds. We placed the mist nets along a narrow, mowed path between the crop fields and the prairie, within the corn and soybean fields, and in the prairie within 10 m of the crop edge. Because we could not remove or trim plants in the cropland or prairie, we placed the nets opportunistically wherever they would not become entangled in vegetation. As a consequence, mist-netting effort varied across locations and we cannot draw conclusions about bird densities based on mist-netting data.

We placed each captured bird into a new brown paper bag for no more than 30 minutes (generally much less time) until it defecated, then collected the fecal sample in 90% ethyl alcohol (EtOH) and placed it on ice in an insulated cooler. We then banded the bird, collected standard measurements and demographic data, and released it. Once out of the field for the day, we stored the fecal samples at -20 °C.

2.3.4 DNA Meta-barcoding Analysis of Fecal Samples

Fecal DNA samples were analyzed using meta-barcoding, a technique that can determine diet composition from fecal samples with a high degree of taxonomic specificity. We

homogenized each raw fecal sample using a FastPrep-24 5G Homogenizer (MP Biomedicals) and extracted DNA using PowerSoil DNA Isolation Kits (MoBio). We amplified DNA with polymerase chain reactions (PCR) using the LCO1490/HCO2198 primers (Folmer et al. 1994; Hebert et al. 2003, see Appendix A in Supporting Information for PCR conditions). DNA Sequencing was performed on an Illumina MiSeq instrument employing V3 chemistry. The LCO/HCO primers give a 710 bp amplicon, which is too large for paired-end read merging on this instrument. Therefore, we used a set of filters and trim steps to increase the quality of the data used for analysis and annotation of the sequenced regions. First, ambiguous nucleotides were trimmed from the ends, and all reads with internal ambiguous nucleotides were discarded. Primer sequences were then trimmed from either the forward or reverse reads, and any read lacking either sequence was discarded. Subsequently, data were trimmed using a quality threshold of p=0.01, and sequences shorter than 200 bp were discarded. We also discarded all sequences from fecal samples with fewer than 1000 total reads, or where less than 10% of reads passed quality checking. The remaining 200+ base fragments were analyzed using a QIIME pipeline for clustering, annotation, and biological observation matrix formation (Caporaso et al., 2010).

We generated operational taxonomic unit (OTU) clusters denovo using the UCLUST method with a 97% sequence similarity threshold. Taxonomic annotations for each OTU were determined using a BLAST search of the NCBI nt nucleotide database (Benson et al., 2012), and used only OTUs identified to the species level in our subsequent analyses. The results from the BLAST search were then processed using the program MEGAN to generate the taxonomic consensus at each taxonomic level (Huson et al., 2007). Because we did not use a sterile technique in the field while collecting fecal samples, we expected our samples to be

contaminated with bacterial, fungal, and human DNA (although we used new materials for each sample to avoid cross-contamination). Although our primers are arthropod-specific, non-arthropod DNA may still be found in samples after PCR. Therefore, we then discarded all OTUs that were not placed in either class Arachnida or Insecta, and samples with fewer than 100 reads assigned to phylum Arthropoda. Because we were interested in species likely to have been directly consumed as prey by birds, we further narrowed our dataset to include only Arachnids in the order Araneae (spiders). This allowed us to exclude species such as feather mites that were present but not of interest in this study.

We compared the taxonomic lists of bird diet components produced by the DNA analysis to lists of arthropod pests of field crops in the Illinois Field Crop Scouting Manual (Bissonnette, 2010). We identified arthropods as potential economically-significant pest species if they appeared in this manual, even if they are generally pests of field crops other than corn or soybeans (e.g. alfalfa) because these field crops are all often grown in close proximity in Illinois. All pests listed in the manual have the potential to cause yield-reducing ("economically-significant") damage to field crops. We were further able to assign the main crop affected by these pests based on recommendations within the Scouting Manual: some pests are known to cause economically-significant damage to corn, soybean, or other crops, while generalist pests may affect multiple crop types. We also assigned general feeding guilds to each arthropod species detected, using Triplehorn and Johnson (2005) as a guide, and The Encyclopedia of Life (Parr et al. 2014) when further information was needed. We assigned arthropods to the following guilds: herbivores, omnivores, natural enemies (predators and parasitoids), and other (for detritivores and species that fall into multiple feeding guilds during different life stages).

We cannot reliably determine the proportional components of an individual bird's diet due to the biases introduced in PCR and the metabarcoding process (Jedlicka et al., 2017), so we instead calculated the percentages of fecal samples that contained DNA from various arthropod species. We normalized sequence read counts by dividing the number of reads per OTU by the total count of OTUs assigned to phylum Arthropoda for each sample. We considered species to be present if they represented at least 1% of the reads per sample (*sensu* McInnes *et al.* 2017), and at least 5 reads per OTU. This may be considered a fairly conservative approach to assigning presence: Deagle et al. (2019) suggests that a 1% presence threshold is suitable for many situations except where the diet is extremely diverse, in which case a much lower threshold is warranted. Because we were interested in the collective effects of the bird assemblage, we pooled data from fecal samples across all bird species for the majority of our analyses, but also compared dietary components from bird species from which we were able to obtain at least ten fecal samples.

2.3.5 <u>Exclosure Data Analysis</u>

We conducted separate analyses for corn and soybean crops. We checked response variables (grain yield per plant) for normality using Shapiro-Wilk tests (Shapiro and Wilk, 1965). Because these variables were normally distributed, we fit linear mixed effects models and used F-tests to check for an effect of exclosure treatment, distance from field edge, and a treatment×distance interaction on total grain yield. We modeled exclosure/control replicates and plant replicates within exclosures/control plots as nested random effects, and exclosure treatment, distance, and the treatment×distance interaction as fixed effects. We included these nested random effects in our models to account for the lack of independence among sampled plants within exclosures and matching controls (Harrison et al., 2018; Millar and Anderson,

2004). We ran a single full model for each crop type (including random effects and treatment, distance, and interaction fixed effects). We then applied a stepwise approach, and removed any nonsignificant predictor variables (P>0.05 or 95% confidence intervals overlap 0) and re-ran the model with only significant predictor variables. We used the coefficient of the treatment effect from the final model (including only significant predictor variables) to calculate the economic value of bird effects as described below. All exclosure analyses were conducted with R version 3.4.3 (R Core Team, 2019).

2.3.6 Economic Value of Bird Effects

To calculate the approximate economic value per hectare of bird-provisioned services and disservices, we applied the average estimated differences in crop yield between exclosures and control plots for both corn and soybeans to equation 1:

$$\frac{\Delta grain \ yield \ (g)}{plant} \times \frac{lb}{g} \times \frac{bushel}{lb} \times \frac{\$}{bushel} \times \frac{plants}{acre} \times \frac{acre}{hectare} = \frac{\$}{hectare} \tag{1}$$

Because field corn and soybeans are generally not grown for direct human consumption in this agroecosystem, we used the dry mass of crop grain yield in this equation. We did not account for cosmetically damaged crops as this generally does not decrease crop value. We used the average grain price per bushel from Illinois during the 2016 marketing year (USDA: NASS 2018) and average planting densities obtained from the farmers of our study site (values differ by crop type and are listed in Table I, Appendix B).

2.4 <u>RESULTS</u>

2.4.1 Exclosures

We found significant, although opposite, effects of exclosure treatment on both corn and soybean grain yield (Table 1). Corn yield was significantly greater in control plots while soybean grain yield was significantly greater in exclosures. Neither distance from field edge nor the interaction between treatment and distance from field edge had a significant effect on yield for either crop type (P > 0.05 and 95% confidence intervals overlap 0), so we removed these predictor variables from our final models (as presented in Table 1).

TABLE I. Final linear mixed effects models of grain yield as a function of exclosure treatment for two crop types. Plant replicate nested within exclosure replicate are modeled as random effects. N=6 exclosure/control plots, 60 plants.

s. iv o exclosure/control plots, oo plants.						
	Estimate	Standard Error	LCI*	UCI*	<i>p</i> -value	
SOYBEANS						
Intercept	11.60	1.14	9.31	13.89	1.13x10 ⁻⁶	
Exclosure treatment	4.05	1.27	1.54	6.57	0.002	
CORN						
Intercept	177.43	20.76	134.17	220.68	1.48 x10 ⁻⁴	
Exclosure treatment	-27.45	12.01	-51.36	-3.54	0.030	

*95% lower and upper confidence intervals for estimate

2.4.2 DNA Metabarcoding Diet Analysis

We amplified arthropod DNA from 113 fecal samples collected from 19 bird species (Table 2). Of the 113 samples, 23 did not have any OTUs that met our criteria for quality, minimum number of reads, and/or taxonomy (see *DNA Meta-barcoding Analysis of Fecal Samples* in Methods). Across all 113 fecal samples, we identified DNA from 61 arthropod species, representing eight orders in Class Insecta and one order in Class Arachnida (Araneae) ($\bar{x} = 2.42 \pm 2.38$ SD species/sample, range = 0–9 species per sample, Table 3).

We detected DNA from six field crop pest species listed in the Illinois Field Crop Scouting Manual: three species that affect corn crops (northern and western corn rootworms, *Diabrotica barberi* and *D. virgifera*; and a sap beetle, *Carpophilus antiquus*), one that mainly affects soybeans (a stink bug, *Euschistus variolarius*), one that generally affects alfalfa (tarnished plant bug, *Lygus lineolaris*), and one generalist species that affects many crop types (a grasshopper, *Encoptolophus costalis*; Figure 3). We also detected DNA from at least one species of natural enemy arthropod in 22.1% of fecal samples. We generally detected more species of herbivorous arthropods than arthropods of other feeding guilds per sample (Figure 4). Northern corn rootworm (detected in 34.5% of samples) was the most commonly detected of the corn or soybean pest species, while spiders (order Araneae, at least one species detected in 19.5% of samples) were the most commonly detected natural enemy (Table 3).

We obtained at least ten fecal samples each from four bird species (Table 2). Of those species, Song Sparrows (*Melospiza melodia*) had the highest percentage of samples that contained DNA from northern corn rootworms (Figure 5A) and also tarnished plant bugs (Figure 5B), the two most commonly encountered pests in our diet study.

2.4.3 <u>Economic Value of Bird Effects</u>

Birds provided a service worth approximately US \$275/ha in the corn crop, and a disservice valued at approximately US \$348/ha in the soybean crop. These extrapolated values are relevant to crop yield within 55 m from a prairie edge, and do not necessarily apply to agricultural areas farther from prairie if there is a distance threshold at which bird foraging decreases.

		Location where bird was captured*				
Common Namo	Sajantifia Nama	Crop	Crop	Prairie	Prairie	Total
	Scientific Ivanie	Edge	Interior	Edge	Interior	Samples
Common Yellowthroat	Geothlypis trichas	5	2	17	5	29
Song Sparrow	Melospiza melodia	3	2	9	3	17
Gray Catbird	Dumetella carolinensis	7	0	6	0	13
Dickcissel	Spiza americana	1	4	2	5	12
House Wren	Troglodytes aedon	3	0	5	0	8
American Goldfinch	Carduelis tristis	1	2	2	2	7
Field Sparrow	Spizella pulsilla	2	2	3	0	7
American Robin	Turdus migrantorius	1	1	0	1	3
Indigo Bunting	Passerina cyanea	3	0	0	0	3
Red-winged Blackbird	Agelaius phoeniceus	0	2	0	1	3
Savannah Sparrow	Passerculus sandwichensis	2	0	0	0	2
Willow Flycatcher	Empidonax traillii	2	0	0	0	2
Brown-headed Cowbird	Molothrus ater	1	0	0	0	1
Brown Thrasher	Toxostoma rufum	0	1	0	0	1
Downy Woodpecker	Picoides pubescens	0	0	0	1	1
Eastern Kingbird	Tyrannus tyrannus	0	0	1	0	1
Eastern Meadowlark	Sturnella magna	0	1	0	0	1
Eastern Towhee	Pipilo erythrophthalmus	1	0	0	0	1
Northern Flicker	Colaptes auratus	1	0	0	0	1
	Totals:	33	17	46	18	113

TABLE II. Quantity of fecal samples collected and dna successfully sequenced from 19 bird species.

*Mist net effort was unequal between locations. Crop edge includes corn or soybeans within 5 m of the cultivated field edge. Crop interior includes corn or soybeans >5 m from the cultivated field edge. Prairie edge includes prairie habitat within 5 m of the cultivated field edge. Prairie interior includes prairie habitat 5–10 m from the cultivated field edge

TABLE III. Arthropod species detected in bird fecal samples through dna barcoding, and their general feeding guilds; n=113 fecal samples. Species in bold were detected in at least 5% of the fecal samples.

ORDER	FAMILY	GENUS	SPECIES	FEEDING GUILD*	COUNT SAMPLES	PERCENT SAMPLES
ARANEAE	Araneidae	Argiope	trifasciata	natural enemy	1	0.9%
	Clubionidae	Clubiona	abboti	natural enemy	7	6.2%
	Corinnidae	Trachelas	tranquillus	natural enemy	2	1.8%
	Linyphiidae	Diplostyla	concolor	natural enemy	1	0.9%
		Grammonota	angusta	natural enemy	1	0.9%
	Lycosidae	Pardosa	milvina	natural enemy	6	5.3%
		Trochosa	ruricola	natural enemy	1	0.9%
	Salticidae	Phidippus	clarus	natural enemy	2	1.8%
		Tutelina	similis	natural enemy	2	1.8%
	Tetragnathidae	Pachygnatha	autumnalis	natural enemy	1	0.9%
	Theridiidae	Theridion	frondeum	natural enemy	2	1.8%
	Thomisidae	Ozyptila	praticola	natural enemy	1	0.9%
		Xysticus	ferox	natural enemy	1	0.9%
COLEOPTERA	Brentidae	Perapion	curtirostre	herbivore	1	0.9%
	Carabidae	Bembidion	quadrimaculatum	natural enemy	1	0.9%
		Pterostichus	melanarius	natural enemy	1	0.9%
	Chrysomelidae	Diabrotica	barberi	herbivore	39	34.5%
		Diabrotica	virgifera	herbivore	1	0.9%
		Epitrix	fasciata	herbivore	1	0.9%
	Curculionidae	Larinus	planus	herbivore	1	0.9%
		Rhinoncus	castor	herbivore	1	0.9%
	Nitidulidae	Carpophilus	antiquus	herbivore	2	1.8%
	Silphidae	Ptomascopus	morio	other	10	8.8%
DIPTERA	Tephritidae	Rhagoletis	cingulata	herbivore	2	1.8%
	Agromyzidae	Liriomyza	brassicae	herbivore	1	0.9%
	Chloropidae	Malloewia	sp.	other	1	0.9%
	Drosophilidae	Drosophila	melanogaster	other	1	0.9%
	Calliphoridae	Pollenia	pediculata	other	25	22.1%
	Chironomidae	Orthocladius	oblidens	other	1	0.9%
	Limoniidae	Helius	flavipes	other	1	0.9%
		Limonia	novaeangliae	other	1	0.9%
HEMIPTERA	Aphididae	Capitophorus	elaeagni	herbivore	1	0.9%
		Chaitophorus	nigrae	herbivore	1	0.9%
	Cicadidae	Tibicen	lyricen	herbivore	1	0.9%
	Coccidae	Parthenolecanium	corni	herbivore	3	2.7%
	Dictyopharidae	Nersia	florida	herbivore	3	2.7%
	Miridae	Lygus	lineolarus	herbivore	44	38.9%
		Neurocolpus	sp.	herbivore	1	0.9%

TABLE III (CONTINUED)

HEMIPTERA	Pentatomidae	Euschistus	variolarius	herbivore	15	13.3%
(CONTINUED)	Alydidae	Alydus	sp.	herbivore	1	0.9%
	Lygaeidae	Neortholomus	scolopax	herbivore	1	0.9%
	Rhyparochromidae	Ligyrocoris	sylvestris	herbivore	1	0.9%
HYMENOPTERA	Agaonidae	Ceratosolen	n. sp.	herbivore	1	0.9%
	Cynipidae	Antistrophus	silphii	herbivore	1	0.9%
	Formicidae	Lasius	alienus	omnivore	28	24.8%
		Lasius	neoniger	omnivore	23	20.4%
		Camponotus	pennsylvanicus	omnivore	1	0.9%
		Stenamma	wheelerorum	omnivore	1	0.9%
	Scelionidae	Telenomus	podisi	natural enemy	1	0.9%
	Tenthredinidae	Caliroa	fasciata	other	1	0.9%
LEPIDOPTERA	Geometridae	Eulithis	sp.	herbivore	3	2.7%
		Eupithecia	miserulata	herbivore	2	1.8%
	Noctuidae	Apamea	sordens	herbivore	1	0.9%
		Galgula	partita	herbivore	1	0.9%
	Pterophoridae	Emmelina	monodactyla	herbivore	1	0.9%
	Crambidae	Neodactria	sp.	herbivore	1	0.9%
ORTHOPTERA	Acrididae	Encoptolophus	costalis	herbivore	1	0.9%
	Gryllidae	Eunemobius	carolinus	herbivore	1	0.9%
		Gryllus	pennsylvanicus	herbivore	1	0.9%
THYSANOPTERA	Thripidae	Frankliniella	tritici	herbivore	13	11.5%
TRICHOPTERA	Hydropsychidae	Potamyia	flava	other	1	0.9%

*Feeding guilds determined from Triplehorn and Johnson 2005. "Natural enemy" includes predators and parasitoids; "other" includes detritivores and species that are in different feeding guilds during different life stages.



Figure 3. Percent of fecal samples (from all bird species) containing DNA from arthropod pests of field crops (see text for explanation of pest criteria); *n*=113 fecal samples. Arthropod pests are grouped by the primary crop type affected by the pest species; "other" category includes multiple crop types or field crops other than corn or soybeans. Genera indicated are: *Carpophilus, Diabrotica, Encoptolophus, Euschistus, Diabrotica, and Lygus.*



Figure 4. Categorized histogram of the number of arthropod species detected per bird fecal sample, grouped by arthropod feeding guild; *n*=113 fecal samples. "Natural Enemy" category includes predators and parasitoids. "Other" category includes detritivores, as well as species that change feeding guild between life stages.



Figure 5. Number of bird fecal samples containing DNA from (**A**) the corn pest *Diabrotica barberi* (northern corn rootworm), and (**B**) the alfalfa pest *Lygus lineolarus* (tarnished plant bug) from the four bird species with at least 10 fecal samples. Bird species indicated are *Spiza americana* (Dickcissel), *Dumetella carolinensis* (Gray Catbird), *Melospiza melodia* (Song Sparrow), and *Geothlypis trichas* (Common Yellowthroat). Percentages in bars indicate the percent of fecal samples per bird species in which pest DNA was present.

2.5 **DISCUSSION**

This study is the first to demonstrate that birds can provide substantial services in field corn, and disservices in soybean crops. We documented a higher corn yield, and a lower soybean yield, when birds were allowed access to crops. DNA diet analysis showed that many birds captured in experimental fields and nearby prairie consumed an economically-significant pest of corn, northern corn rootworm (34.5% of samples), and both predatory spiders (Araneae, 19.5% of samples) and predatory beetles (*Carabidae*, 1.8% of samples). The fact that many individual birds consumed corn pests that can cause significant economic damage may explain the net positive services provided by birds in the corn field. Conversely, only 13.3% of fecal samples contained DNA from an economically-significant soybean pest (Figure 3). The net disservices in soybeans may be due to birds consuming natural enemy arthropods that would otherwise provide biological control, while rarely consuming the pests themselves. Furthermore, bird disservices may be more pronounced than services if birds eat predatory arthropods that naturally occur in lower densities than herbivores. Together, these results suggest that birds have the potential for more substantial effects on conventionally grown field crops than previously expected.

Pests of field crops vary by year and by crop type. While corn rootworms are the main corn-specific pest that birds consumed in our study system, tarnished plant bugs (*Lygus lineolarus*) were detected in more fecal samples than any other pest species (Figure 3). Although the crop scouting manual lists tarnished plant bugs as pests of alfalfa crops, these bugs can make use of an extremely wide variety of plant hosts, including soybeans (Snodgrass et al., 2010). Bird consumption of tarnished plant bugs also may have consequences across the larger landscape because alfalfa fields are common within the agricultural matrix in Illinois and much of the Midwest of the United States.

In many systems, pest removal services may be driven disproportionately by a single or a few species of predator (Letourneau et al., 2009; Maas et al., 2015). Of the four bird species for which we tested at least 10 fecal samples, Song Sparrows had the highest proportion of fecal samples containing DNA from corn rootworm and tarnished plant bug (Figures 5A and B). Song Sparrows are primarily insectivorous during the breeding season, and they can consume a wide range of sizes of arthropod prey (Arcese et al., 2002). Because Song Sparrows are found in a wide variety of habitats and are more generalist in habitat affiliation than many grassland bird species, they have the potential to provide extensive services across many landscapes. Indeed, they are often found along the shrubby edges of agricultural fields. Furthermore, as a resident species across much of its range, the Song Sparrow has potential to consume pests both earlier and later into the growing season than migratory species.

The corn variety grown in our study system was genetically modified (GM) to express Bt toxins, and the soybeans were sprayed with broad spectrum insecticides. These are extremely common pest management strategies for corn and soybeans, respectively, in the United States. Although we do not have data on pest densities before and after insecticide treatment, it is possible that bird services were undervalued in soybean crops due to insecticide use. Nevertheless, we found that bird trophic interactions in this system were strong enough to be detectable despite those pest management strategies. Because some species of corn rootworms are beginning to show resistance to Bt transgenic corn crops (Gassmann et al., 2014), our results take on added significance. We expect that these indirect bird effects may be even stronger in organic crop systems, or those that do not employ chemical or GM-produced insecticides (but see Garfinkel and Johnson 2015).
While studies have found that birds consume corn pests (e.g. Bendell et al. 1981, Bollinger and Caslick 1985), the only other study that quantified bird indirect effects in field corn (Tremblay et al., 2001) found depression of corn pests, but no cascading increase of corn grain yield. Exclosure effects may be highly dependent on many variables including the surrounding landscape (Boesing et al., 2017), and local prey or pest conditions (Halaj and Wise, 2001; Salo et al., 2010), which vary with location, time, and management practices. The disparity of our findings from those of Tremblay et al. (2001) may result from differences in such conditions.

We know of no other study that examined indirect bird effects in soybean crops. Results from our DNA analysis suggest that the disservice in soybeans resulted from intraguild predation of birds on arthropod predators, thus releasing pest species. Birds have also been shown to provide indirect disservices in other systems such as non-maize grain (Grass et al., 2017; Tschumi et al., 2018) and cabbage crops (Martin et al., 2013). Those studies, like ours, suggested that bird disservices were caused by intraguild predation, where birds consumed predatory or parasitoid arthropods.

DNA metabarcoding of scat is an evolving, minimally invasive technique that provides highly specific data on prey identity that would otherwise be difficult to ascertain using older methods of diet analysis (e.g. emetics or stomach sampling; McInnes et al. 2017a). Like many other diet analysis techniques, however, we cannot determine where the bird captured prey items, or the life stage of the prey items consumed. While the sampled birds almost certainly foraged in the prairie, certain prey items, such as crop pests, most likely originated in the crop fields. Moreover, any potential crop pest that is consumed in prairie habitat is no longer able to spill over into the adjacent crop (Tscharntke et al., 2012). Therefore, we believe that identifying prey

species helps explain the mechanisms behind the bird service and disservice provision at the larger multi-field scale.

Although our estimated economic values of bird effects (+\$275/ha in corn, and - US \$348/ha in soybeans) are only approximations, these results suggest that birds provide previously unexpected but substantial economic consequences in conventional agriculture. These net effects deserve further exploration because the perceived costs and/or benefits from wildlife such as birds are among the strongest drivers of farmers adopting conservation practices (Kross et al., 2018). Indeed, new technologies such as precision agriculture may allow farmers in the future to take low-yield areas out of production and replace them with native plantings that encourage birds and their services, and potentially increase their overall crop yield and biodiversity (see Lindell et al. 2018). Because the majority of farmers rotate corn and soybean crops (Wiebe and Gollehon, 2006), it will be important to determine overall economic effects of bird populations over a multi-year rotational schedule. This may be best addressed with models that are beyond the scope of the current paper.

Contrary to our hypothesis, we found that distance from prairie/field edge was not a significant predictor of grain yield or exclosure treatment effect for either crop. This may indicate that our "field interior" exclosures, placed 55 m into the field, had not yet reached an interior threshold where bird foraging declined compared to the field edge, provided that such an edge effect exists. It is also possible that our study fields are not representative of crop fields in terms of size, and larger crop fields (with a smaller edge:area ratio) would show a stronger distance effect. Because of the low levels of replication in our study, future research should expand specifically on both the spatial and temporal scale of our study to determine if these net effects in corn and soybean crops are consistent across space and time. Future studies should also

incorporate within-year changes in bird diets to determine whether net services vs. disservices may differ throughout a growing season (Grass et al., 2017).

Our study provides the first evidence of a previously unquantified, but potentially ecologically and economically important, process within a widespread agroecosystem. Although our study was conducted across a small scale, these results show that bird communities have the potential to produce real economic effects even in large scale, conventional monocrop systems. While we caution using these results to generalize across all similar systems, the substantial effects we found in this study indicate a need for further research to better explicate net bird effects in conventional corn and soybean agriculture.

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2.8 APPENDICES

2.8.1 Appendix A. PCR conditions for amplicon sequencing

Primers without CS tags used for sequencing (Folmer et al. 1994):

LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3'

HC02198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

PCR was run twice to facilitate addition of CS tags (the first time using primers without tags, and the second time with the tags). The gDNA samples from fecal DNA extraction were diluted 1:10 due to the presence of inhibitors in the samples. PCR was not replicated and products were pooled (see Smith and Peay 2014 for a discussion of a study indicating that PCR replication did not alter observed ecological data). The PCR conditions were as follows:

First PCR using primers without CS tags:

01		U
Temperature	Time	Number of Cycles
95° C	3 min.	
95° C	1 min.	
40° C	1 min.	27
72° C	1.5 min.	
4° C	œ	

Second PCR using primers with CS tags:

Temperature	Time	Number of Cycles
95° C	3 min.	·
95° C	1 min.	
40° C	1 min.	8
72° C	1.5 min.	
4° C	œ	
1.6	00	

CITED WORK:

Smith, D.P., Peay, K.G., 2014. Sequence Depth, Not PCR Replication, Improves Ecological Inference from Next Generation DNA Sequencing. PLoS ONE 9, e90234. https://doi.org/10.1371/journal.pone.0090234

2.8.2 Appendix B

Value	Corn	Soybeans
US \$/bushel, IL, 2016 marketing year ¹	3.43	9.78
Pounds/gram	0.0022	0.0022
bushels/pound ¹	0.0179	0.0167
treatment effect (grams/plant) ²	27.45	-4.05
Average number plants/acre ³	30,000	100,000
Acres/hectare	2.47	2.47

Table I. Values used in Equation 1 to perform economic calculations

¹From USDA: National Agricultural Statistics Service 2018

²Values from bird exclosure treatment effects reported in this study

³Information provided by farmers of study fields

3. WHEN A PEST IS NOT A PEST: BIRD INTRAGUILD PREDATION INCREASES SOYBEAN PEST LEAF DAMAGE BUT DOES NOT DECREASE CROP YIELD

3.1. <u>ABSTRACT</u>

Ecosystem disservices, those aspects of an ecosystem that can have negative impacts on humans, may disproportionately affect conservation decisions made by farmers. Therefore, it is important to quantify them in a meaningful way. This is particularly true in cases where a perceived disservice may not actually have a measurable economic impact. For instance, pest damage to crops does not always decrease crop yield if the plant can compensate with regrowth. When birds consume beneficial predatory arthropods rather than pest species (intraguild predation), they can provide a disservice to the farmer, but only if the intraguild predation affects crop yield. We studied bird intraguild predation in northern Illinois (USA) at six soybean fields adjacent to grasslands which provided source habitat for bird populations. We placed cages over soybean crops, which excluded birds but allowed access to arthropods, and measured differences in leaf damage due to pests and overall crop yield between control and exclosure plots. We found that exclosure plots had lower levels of leaf damage by pests than control plots, but there was no resulting effect on crop yield. We also found that sites with higher bird species richness had overall lower levels of leaf damage by pests. These results suggest that although birds may have released pests through intraguild predation, there was no net disservice when considering crop yield, the variable most important to stakeholders.

3.2. INTRODUCTION

The valuation of ecosystem services has been an important approach to incentivizing biological conservation, particularly of birds and their habitats (e.g. Sekercioglu et al. 2016, Wilsey et al. 2016). The valuation of ecosystem disservices, however, may be equally if not more important to conservation, because many stakeholders make their decisions based on losses

rather than gains (Kross et al. 2018, Blanco et al. 2019). This is especially significant in cases where a perceived disservice may not actually have a measurable economic impact. For instance, Borkhataria et al. (2012) found that although blackbirds directly damaged rice crops, this did not reduce the average crop yield. In fact, most agricultural crop plants can withstand pest damage with no effect on crop yield until they reach an economic injury level, after which point crop yield declines (Pedigo et al. 1986).

While birds often provide important pest control services in agricultural settings (Whelan et al. 2016a), they can also provide disservices in a variety of ways. Most research on agricultural ecosystem disservices by birds examines direct effects such as crop consumption or damage, or decreased food safety due to pathogens spread by birds (Pejchar et al. 2018). Indirect disservices caused by birds are harder to measure than direct ones, but still have the potential to cause measurable and important effects on agroecosystems. Birds can provide an indirect disservice when they consume beneficial arthropods such as pollinators (Knight et al. 2006) or predatory arthropods that would otherwise consume pest species (i.e. intraguild predation; Garfinkel et al. 2020). However, just as low levels of herbivory may not affect crop yield, low levels of intraguild predation might also not negatively impact yield. Whether intraguild predation by birds is a real disservice, and not just a perceived one (e.g. Basili and Temple 1999), depends on the ultimate effect on crop yield.

Integrated pest management (IPM) is an approach to agricultural pest control that greatly increases farmland resiliency while providing biological conservation benefits (Barzman et al. 2015). Under an IPM approach, farmers may use a combination of chemical, cultural, mechanical, and biological control techniques to keep pest damage below the economic injury level (Stenberg 2017). Low pest densities that cause damage but have no effect on crop yield are

tolerated under this approach. Similarly, birds that consume predatory arthropods below a level that affects crop yield would also be tolerated. Because birds also have the potential to simultaneously provide services by consuming pest insects, they can be valuable for farmers using IPM (Garfinkel et al. 2020). Disentangling the causes of net positive or negative bird effects within agricultural systems may therefore be an important way to incentivize conservation of birds and their nearby habitats within the agricultural matrix.

Many studies of bird trophic effects in agriculture take place in orchards, vineyards, or agroforestry systems (Koh 2008, Johnson et al. 2010, Crisol-Martínez et al. 2016, Mangan et al. 2017), perhaps because the complex vegetative structure provided by trees and shrubs provides good habitat for birds (Rodenhouse and Best 1983, Erdelen 1984, Wilson et al. 2017). Studies that examine bird trophic effects in row crop agriculture often take place in small scale, organic, or otherwise "wildlife friendly" agroecosystems where bird populations may be larger (e.g. Jones and Sieving 2006, Garfinkel and Johnson 2015, but see Kross et al. 2016, Garfinkel et al. 2020, Olimpi et al. 2020). Although row crop agriculture is grown extensively worldwide, few studies have examined bird trophic effects in these systems (e.g. Tremblay et al. 2001, Borkhataria et al. 2012).

Soybean crops are an ideal system in which to study bird trophic interactions in large-scale conventional row-crop agriculture for several reasons. First, soybeans are consistently one of the top commodities in the US, with over 3.7 million hectares of land planted in 2017 (National Agricultural Statistics Service 2017). Second, soybeans are vulnerable to a variety of arthropod crop pests of varying sizes and taxonomic orders (Bissonnette 2008). Finally, although soybeans are generally grown in large monocrop fields, differing habitat surrounding the fields may allow us to compare trophic effects in landscapes with increased bird diversity. For instance, in the

Midwest of the United States where the majority of soybeans are grown (National Agricultural Statistics Service 2017), remnant and restored prairies and grasslands are among the dominant "natural" habitat types, and often may be found near cultivated land. We might expect to find more intraguild predation by birds in fields close to natural habitats, because these areas may show both increased bird foraging activity (Rodenhouse and Best 1994, Puckett et al. 2009) and increase density of predatory arthropods (Macfadyen and Muller 2013), although they may harbor higher pest densities as well (Nguyen and Nansen 2018).

In a previous study of agricultural fields next to grassland habitat (Garfinkel et al. 2020), we found that birds provided indirect services in a corn field but disservices in a soybean field. The goal of the current study was to determine whether those disservices provided by birds in soybeans grown adjacent to grassland were consistent among sites. We used bird exclosures over soybean crops to measure the indirect effect of birds on soybean leaf damage and total crop yield. We hypothesized that there would be a more pronounced bird effect in sites with higher bird biodiversity or abundance, and along the edges of the field closer to grassland habitat. This study will clarify the variables that affect the net ecosystem services by birds and therefore help farmers make educated decisions that maximize crop yield without negatively affecting nearby bird habitats.

3.3. METHODS

3.3.1. Study Sites

We conducted our study at six sites in Kane, DeKalb, and Ogle counties in northern Illinois, USA. We selected sites that had a soybean field which shared at least one field edge with a grassland or prairie, and where we were able to obtain permission from all involved landowners

to conduct our research. Each site was separated from others by at least 1km. The average size of the soybean fields was 23 ha (range = 4 - 42 ha).

3.3.2. Exclosures

At each of the six sites, we placed four bird exclosures over soybean crops in mid-June in 2017. Each exclosure was paired with a marked control plot 2m away. The exclosure array was centered along the crop field edge that shared a border with the prairie or grassland. Two exclosure/control pairs were placed 5m into the crop field from the field edge and 50m from each other, and the other two were 55m into the field interior from the field edge and 50m from each other (Figure 6).



Figure 6. Experimental configuration at each of six study sites. Exclosure plots are represented by rectangles surrounded by solid black lines, and control plots are represented by rectangles surrounded by dotted black lines. The star represents the approximate location where observers stood for point counts. Arrows highlight the distances between plots, and exclosures and control plots are not drawn to scale.

We constructed the exclosures from PVC pipe frames covered with 2.5cm square (5cm stretch) monofilament netting (Memphis Net and Twine Company, Tennessee, USA). We chose this netting size to be small enough to exclude all birds, but large enough to allow access by most arthropods. Previous studies have used even finer mesh over soybeans and found no direct effect of the exclosure itself on plant growth (see Costamagna et al. 2007), suggesting that any differences in crop yield between control and exclosure plots should only be due to bird exclusion. Each exclosure was 0.6m wide (which fit over one or two rows of plants depending on row spacing), 1.5m long, and 1.5m tall. Although each exclosure covered differing numbers of soybean plants, we marked the central five plants in the exclosure with small plastic plant tags for future measurements.

We placed the exclosures over crops once they were established in the field, but before they started flowering. Once deployed, we left the exclosures in place for the rest of the growing season and removed them once the crops were dry and ready for harvest.

We scored insect damage to soybean leaves three times throughout the growing season: 1) immediately before the exclosures were placed, 2) when the crops were just beginning to bloom (also known as growth stage R1, [Licht 2014]), and 3) when the crops were beginning to develop seed pods (growth stage R5). Ten haphazardly selected leaf triads were selected from each exclosure and control plot, and we scored insect defoliation of each triad to the nearest 10% using a visual guide developed by University of Minnesota Extension (Koch 2016). At each visit, we selected the leaf triads to be scored, therefore likely scoring different leaves at each time point. Upon removing the exclosures at the end of the growing season, we hand-harvested soybean seed pods from the five marked plants in each exclosure and control plot, oven dried the

soybeans after removing them from the pods, and recorded the dry mass and count of soybeans from each focal plant.

3.3.3. Point Counts

One of us (MG) with one other experienced assistant conducted bird point counts together at each site, once in May and once in July 2017, for a total of two counts per site. The point counts were conducted between sunrise and 9am, and only in appropriate weather conditions (no rain or strong wind). The observers were positioned on the border between grassland and cropland at each site, equidistant from the two nearest exclosures (see Figure 6). Each point count lasted 10 minutes. For each bird heard or seen within the 10-minute point count, the observers recorded species and habitat use (cropland or non-cropland habitat). If a species was detected immediately before or after the 10-minute point count but not also detected during the point count, the observers recorded the species (marked as outside of the point count), but not location or habitat. These species were included in richness (Buskirk and McDonald 1997) but not abundance measures of bird populations. From the pooled data from the two point counts per site, we calculated the following bird population metrics for each site: total species richness, species richness of birds detected on cropland habitat, total bird abundance, and abundance of birds detected on cropland habitat.

3.3.4. Analysis

We modeled the effect of exclosure treatment on both leaf damage and crop yield. We separated out the leaf damage scores into pre- and post-exclosure treatment: pre-treatment measurements were taken immediately before exclosure placement, and post-exclosure treatment measurements were taken at growth stages R1 and R5 as described above. We pooled both post-treatment leaf damage measurements into a single data set for analysis, and included a variable

indicating at which growth stage the measurement was taken. This gave us a total of three response variables which we modeled in separate analyses (pre-treatment leaf damage, post-treatment leaf damage, and crop yield). The leaf damage response variables were calculated from the damage scores averaged per plot; crop yield was calculated as the total grams of dried soybeans produced per plot. We conducted separate analyses for pre- and post-treatment leaf damage instead of a single repeated measures analysis because we haphazardly chose leaves to assess each of the three times we measured leaf damage; as we did not follow the growth of individual leaves throughout the growing season, we likely measured different leaves each time. Although we averaged leaf damage per plot, the soybean plants were very small pre-treatment, and we often had to measure all leaves within the plot to reach 10 measured leaf triads. By comparison, the plants were much larger during the post-treatment measurements, and 10 leaves per plot was only a small subset of the available leaves. Therefore pre- and post-treatment leaf damage measurements were not entirely comparable, and we opted to analyze them separately.

First, we ensured that all three response variables were either normally distributed, or log transformed them to normal as confirmed with Shapiro-Wilk tests (Shapiro and Wilk 1965). We created linear mixed effect models with the normal or lognormal data, and compared our models using Akaike's Information Criterion corrected for small sample sizes (AICc, Anderson 2008). In order to decrease the overall number of models run per response variable, we used a step-down approach: first we compared an a priori set of four models per response variable, where each model included one of the four bird population variables (as described above) as the lone fixed effect predictor variable. Next, we chose the bird population variable that best fit the data according to AICc model selection and included that variable alone and in combination with other fixed effect variables in a second model set.

All three AICc model sets (for each of the three response variables) included models with treatment (exclosure or control), plot location (field edge or interior), and bird population variables as fixed effects. All models also included site and treatment replicates as random effects. We also created models with interactions between treatment and plot location to test whether distance from field edge affected the strength of the treatment effect, and interactions between treatment and bird population variables (e.g. species richness per site) to test whether the bird populations affected the strength of the treatment effect. Because the post-treatment leaf damage data included average plot damage scores from two separate time periods, we included models with temporal replicate (measurement during growth stage R1 vs R5) as a fixed effect, and interactions between tereatment effect.

3.4. <u>RESULTS</u>

3.4.1. Point Counts

We detected a total of 55 bird species across both point counts at all sites (mean=23.8 species, SD=5.2, per site). Of the 55 species, 16 were detected in cropland habitat (mean= 5.0 species, SD=2.8 per site, Table I, Appendix C). Total species richness ranged from 19-33 species per site, and species richness of birds detected in cropland ranged from 2-9 species per site (Table IV).

TABLE IV. Bird population measurements summed from two point counts per site. Each bird encountered during the point count was identified as using cropland habitat or other habitat, and crop species richness and abundance refers only to birds detected within cropland habitat.

Site	Total Species Richness	Total Abundance	Crop Species Richness	Crop Species Abundance
А	33	85	2	2
В	19	83	7	28
С	25	93	9	22
D	19	83	5	5
Е	24	70	5	15
F	23	55	2	4

3.4.2. Exclosure Effect on Soybean Leaf Damage

All leaf damage that we recorded appeared in patterns typically caused by arthropod herbivores, as illustrated in a visual guide to soybean defoliation (Koch 2016). Of the four bird population variables, total species richness per site was the best predictor of leaf damage score both before and after treatment; we therefore used this bird population index in our subsequent model sets. Our top models in both the pre- and post-treatment AICc analyses were >2 Δ AIC from the next best ranking model (Table II, Appendix C), so we present here only the results of the top model in each leaf damage analysis.

Exclosure treatment was a significant predictor of leaf damage in the post- but not pretreatment analysis. Post-treatment, exclosures showed significantly lower damage scores than control plots (p < 0.01, Figure 7A). The final top model of leaf defoliation both pre- and posttreatment also included bird species richness and distance from field edge as significant predictor variables (Table V). Lower bird species richness was correlated with increased leaf damage (Figure 8), and field interior plots had significantly lower damage scores than field edge plots (Figure 9).



Figure 7. Boxplots showing A) the average leaf damage score after exclosures were placed by treatment and B) the total dry mass of grain yield per plot by treatment.



Figure 8. Average soybean leaf damage per plot as a function of bird species richness. Line and shaded 95% confidence interval were predicted from the most parsimonious model of leaf damage post-treatment for exclosure plots 5m from the field edge. Points and standard deviation error bars are actual average leaf damage scores for exclosure plots 5m from the field edge per site.



Figure 9. Boxplots showing the average soybean leaf damage post-treatment for control and exclosure plots at two distances from the field edge shared with grassland. Edge plots were 5m from the field edge, and interior plots were 55m from the field edge.

TABLE V. Results of top model for each of three response variable analyses. Defoliation pretreatment measurements are the average leaf damage score per plot before exclosures were placed. Defoliation post-treatment measurements are the average leaf damage score per plot measured at two time periods after exclosures were placed. Crop yield is the total dry mass of grain yield per plot (g).

Response Variable /	Estima	SE	df ^a	n valua	95% CI	
Predictor Variable	te	SE	ui	<i>p</i> -value		
Defoliation Pre-treatment						
Intercept	1.75	0.53	24	0.003	(0.66, 2.84)	
Distance (Interior) ^b	0.46	0.20	24	0.030	(0.05, 0.87)	
Bird Richness: all habitats ^c	-0.05	0.02	24	0.028	(-0.09, -0.01)	
Defoliation Post-treatment						
Intercept	2.12	0.22	23	< 0.001	(1.67, 2.57)	
Treatment (Exclosure) ^d	-0.26	0.07	66	< 0.001	(-0.4, -0.13)	
Distance (Interior)	-0.28	0.08	22	0.002	(-0.45, -0.12)	
Bird Richness: all habitats	-0.02	0.01	22	0.001	(-0.04, -0.01)	
Crop Yield						
Intercept	55.68	7.07	22	< 0.001	(41.17, 70.17)	
Bird Richness: cropland	2.39	1.30	22	0.078	(-0.26, 5.05)	

^{*a*} Denominator degrees of freedom

^b Plot distance is either field edge (5m from field edge) or field interior (55m from field edge) ^c Total bird species richness across all habitat types within each site was used for all response variables except for crop dry mass, where bird species richness within cropland habitat was used because it provided a better fit to the data (see text for explanation of AICc modelling methods) ^d Treatment is exclosure vs. control plot

3.4.3. Exclosure Effect on Soybean Crop Yield

Species richness of birds in cropland habitat (Table IV) was the best predictor of crop yield when compared to the other three bird population variables. We therefore used this bird population index in our subsequent model set. The best model (lowest AICc) in our subsequent model set included species richness of birds in cropland habitat as the only predictor variable of crop yield (Table V). However, this model performed only marginally better than the null model (Δ AICc = 0.62), and the results of that model suggest that species richness was not in fact a significant predictor of crop yield (*p*=0.078, Table V).

3.5. DISCUSSION

Our findings emphasize the importance of measuring crop yield rather than simply plant damage when assessing trophic cascades in agricultural systems, as the two are not always equivalent. We found that excluding birds from plants resulted in lower arthropod pest damage to leaves but did not affect grain yield (Figure 8). This leaf damage is likely due to intraguild predation of birds on predatory arthropods (Garfinkel et al. 2020); birds consuming predatory arthropods such as spiders and carnivorous beetles indirectly release arthropod pests, which then cause damage to the crop in a typical four-level trophic cascade (Martin et al. 2013, Grass et al. 2017). However, this increased damage did not affect crop yield and therefore did not reach the economic injury level.

When plants experience damage below the economic injury level, they can compensate and show no overall reduction in reproductive output (i.e. grain yield; Pedigo et al. 1986). Soybeans, in particular, can withstand large amounts of leaf defoliation without yield loss because they exhibit delayed leaf senescence following injury. The delayed leaf senescence allows them to compensate for earlier lost leaf area (Abu-Shakra et al. 1978). Therefore, although birds in our system may have indirectly promoted leaf damage through their intraguild predation of arthropod predators, they exerted neutral net effects on crop yield.

In a previous study in a similar agroecosystem (Garfinkel et al. 2020), we found that birds indirectly negatively affected crop yield in soybeans grown adjacent to a prairie patch. The contrasting findings in our two studies are likely due to several factors. First, pest densities can vary widely between years (e.g. Rhainds et al. 2010). If pests were at an overall lower density during the current study than the previous study, then we would expect to see a smaller effect of birds on crop yield. Because we did not measure leaf damage in the previous study, we cannot

compare that parameter between studies. Additionally, pest identity varies between years, with the most economically important pest species varying over both time and space (e.g. Bueno et al. 2013). It is possible that the pests during our previous study were less palatable to birds, causing them to consume more predatory arthropods than herbivorous ones. Finally, soybean crops need adequate growing conditions such as ample water in order to compensate for damage with regrowth via delayed leaf senescence (Haile et al. 1998). In northeast Illinois, the summer of the current study was slightly wetter than that of our previous study (25.2 cm of precipitation in June and July combined in 2016, vs. 28.9 cm in 2017; NOAA National Centers for Environmental information 2020). It is therefore possible that rainfall was sufficient to allow for growth compensation in 2017 but not in 2016 during our earlier study. Regardless, our findings emphasize the need for longer-term research on services and disservices within this study system. Future studies should determine how often yearly net bird effects are positive, negative, or neutral, so we can determine how these trophic interactions affect long-term gains or losses and farmland resiliency (Admiraal et al. 2013).

Although we found that birds appear to have promoted leaf damage, sites with higher bird species richness had overall lower levels of leaf damage (Figure 8). We found no evidence that bird species richness was correlated with the strength of the treatment effect (i.e. treatment × species richness interaction was not a significant predictor). It is important to note that our study shows only the net effect of birds within our study system. That net effect represents the collective foraging activity of a varied bird community, where individuals have the potential to provide both services and disservices, and species differ in their diet and behaviors. Therefore, increased bird species richness may be correlated with lower overall damage (despite bird intraguild predation on beneficial arthropods) if species-rich communities are more likely to

include birds that consume more pests than predatory arthropods. Moreover, some studies have shown that species-rich natural enemy assemblages may complement each other, thus strengthening top-down control (Letourneau et al. 2009). Bird species richness may also be correlated with other factors, such as predatory arthropod species richness or abundance. If that is the case, we suggest that improving habitat for both birds and other taxa surrounding the cropland could lead to an overall decrease in crop damage without exacerbating potential indirect disservices caused by birds consuming predatory arthropods. For instance, Martin et al. (2015) found that more complex landscapes had better biological control of aphid pests than simplified landscapes, despite evidence of intraguild predation by birds.

We found lower overall leaf damage in field interior than in edge plots (Figure 10), which is likely due to the fact that many pests are found in higher densities near field edges (Nguyen and Nansen 2018). However, we did not find a significant treatment × distance interaction effect on leaf damage, so we cannot conclude that bird trophic effects were stronger at the field edge. The trophic interactions that occur in field edges are often more complex than those in field interiors, because in addition to increased pest densities, field edges may exhibit spillover of predatory arthropods from crop into natural habitat, and vice versa (Rand et al. 2006, Macfadyen and Muller 2013), in addition to increased bird activity (e.g. Rodenhouse and Best 1994, Puckett et al. 2009). Future studies should specifically examine movement patterns of species from all trophic levels, from pests to birds, along field edges within a mixed soybean/grassland system. This information may help identify optimal ways to incorporate grassland patches into agricultural matrices to maximize vertebrate and invertebrate natural enemy activity within cropland while maintaining high crop yields. Our study raises the question: when is a pest a pest? If we define a pest "as any organism that decreases fitness, population size, growth rate, or economic value of any resource important to humans" (Whelan et al. 2016b), then we suggest that, in agricultural systems, herbivorous arthropods are pests only when their herbivory reduces crop yield. In the present study, although bird predation of arthropod predators appeared to release crop pests, this predatory activity of birds did not cause a reduction in crop yield, the variable of paramount importance from the viewpoint of the farmer. Our study thus illustrates the critical importance of quantifying net effects in agricultural systems when assessing the roles of birds in these systems.

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3.8. <u>APPENDIX C</u>

Appendix TABLE I. Bird S	pecies detected across two p	point counts at each of six study sit	tes.
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Common Name	Scientific Name	No. of Sites ^a	Cropland? ^b
American crow	Corvus brachyrhynchos	4	X
American goldfinch	Spinus tristis	5	Х
American redstart	Setophaga ruticilla	1	
American robin	Turdus migratorius	6	Х
Baltimore oriole	Icterus galbula	2	
Barn swallow	Hirundo rustica	5	Х
Black-billed cuckoo	Coccyzus erythropthalmus	1	
Black-capped chickadee	Poecile atricapillus	1	
Brown-headed cowbird	Molothrus ater	6	Х
Blackburnian warbler	Setophaga fusca	1	
Blue jay	Cyanocitta cristata	2	
Bobolink	Dolichonyx oryzivorus	5	
Brown thrasher	Toxostoma rufum	1	
Canada goose	Branta canadensis	1	Х
Chipping sparrow	Spizella passerina	2	
Chimney swift	Chaetura pelagica	1	
Common grackle	Quiscalus quiscula	2	Х
Common yellowthroat	Geothlypis trichas	6	Х
Dickcissel	Spiza americana	5	Х
Eastern kingbird	Tyrannus tyrannus	2	Х
Eastern meadowlark	Sturnella magna	4	Х
Eastern phoebe	Sayornis phoebe	1	
Eastern towhee	Pipilo erythrophthalmus	1	
Eastern wood-pewee	Contopus virens	1	
European starling	Sturnus vulgaris	3	
Field sparrow	Spizella pusilla	2	
Great blue heron	Ardea herodias	4	
Gray catbird	Dumetella carolinensis	1	
Grasshopper sparrow	Ammodramus savannarum	2	
Henslow's sparrow	Ammodramus henslowii	3	
House wren	Troglodytes aedon	2	
Indigo bunting	Passerina cyanea	4	
Killdeer	Charadrius vociferus	5	Х
Mallard	Anas platyrhynchos	2	
Mourning dove	Zenaida macroura	5	
Northern cardinal	Cardinalis cardinalis	1	
Northern flicker	Colaptes auratus	1	
Northern rough-winged swallow	Stelgidopteryx serripennis	1	
Red-breasted grosbeak	Pheucticus ludovicianus	2	
Ring-necked pheasant	Phasianus colchicus	3	Х
Rock pigeon	Columba livia	1	

Common Name	Scientific Name	No. of Sites ^a	Cropland? ^b
Red-tailed hawk	Buteo jamaicensis	2	
Ruby throated hummingbird	Archilochus colubris	1	
Red winged blackbird	Agelaius phoeniceus	6	Х
Savannah sparrow	Passerculus sandwichensis	3	Х
Sedge wren	Cistothorus platensis	4	
Song sparrow	Melospiza melodia	6	Х
Tree swallow	Tachycineta bicolor	2	
Tufted titmouse	Baeolophus bicolor	1	
Turkey vulture	Cathartes aura	1	
Warbling vireo	Vireo gilvus	2	
Willow flycatcher	Empidonax traillii	3	
Yellow-billed cuckoo	Coccyzus americanus	1	
Yellow warbler	Setophaga petechia	2	

TABLE I (continued)

^aNumber of sites out of 6 total where species was detected at least one time

^bRows marked with an X represent species that were detected at least once on cropland habitat

Appendix TABLE II. AICc rankings of models for three separate predictor variables. Defoliation pre-treatment measurements are the average leaf damage score per plot before exclosures were placed. Defoliation post-treatment measurements are the average leaf damage score per plot measured at two time periods after exclosures were placed. Crop yield is the total dry mass of grain yield per plot (g).

Response Variable/Predictor Variables	\mathbf{K}^1	AICc	ΔAICc	AICcWt	LL^2
Defoliation pre-treatment					
Distance ^a + Richness ^b	6	88.65	0	0.36	-37.3
Richness	5	90.5	1.85	0.14	-39.54
Distance	5	90.8	2.15	0.12	-39.69
Treatment ^c + Richness + Distance	7	91.4	2.75	0.09	-37.3
Null	4	92.16	3.51	0.06	-41.62
Treatment * Richness + Distance	8	92.16	3.51	0.06	-36.23
Treatment + Richness	6	93.11	4.46	0.04	-39.53
Treatment + Distance	6	93.42	4.77	0.03	-39.68
Treatment * Richness	7	93.74	5.09	0.03	-38.47
Treatment * Distance + Richness	8	94.18	5.53	0.02	-37.24
Treatment	5	94.65	6	0.02	-41.61
Treatment * Distance	7	96.06	7.41	0.01	-39.63

Response Variable/Predictor Variables	\mathbf{K}^1	AICc	ΔAICc	AICcWt	LL^2
Defoliation post-treatment					
Treatment + Distance + Richness	7	7	141.66	0.54	-63.13
Rep ^d + Treatment + Distance +	8				
Richness	0	8	143.91	0.18	-63.04
Rep * Treatment + Distance + Richness	9	9	146.06	0.06	-62.88
Rep + Treatment * Richness + Distance	9	9	146.08	0.06	-62.88
Rep + Treatment * Distance + Richness	9	9	146.39	0.05	-63.04
Treatment * Distance	7	7	147.04	0.04	-65.82
Treatment + Distance	7	7	147.05	0.04	-65.82
Rep * Treatment + Distance	8	8	148.95	0.01	-65.57
Rep + Richness	6	6	149.42	0.01	-68.19
Treatment * Richness	6	7	151.47	0	-68.04
Rep + Treatment	7	6	153.06	0	-70.01
Rep * Treatment	6	7	155.09	0	-69.85
Rep + Distance	4	6	156.99	0	-71.97
Null		4	161.14	0	-76.33
Crop Yield					
Richness	5	384.39	0	0.37	-186.41
Null	4	385.01	0.62	0.27	-187.99
Treatment + Richness	6	387.07	2.68	0.1	-186.4
Treatment	5	387.55	3.16	0.08	-187.99
Distance	5	387.56	3.17	0.08	-187.99
Treatment * Richness	7	389.01	4.62	0.04	-185.95
Treatment + Richness + Distance	7	389.91	5.52	0.02	-186.4
Treatment + Distance	6	390.24	5.85	0.02	-187.99
Treatment * Richness + Distance	8	392.01	7.62	0.01	-185.95
Treatment * Distance + Richness	8	392.09	7.7	0.01	-185.99
Treatment * Distance	7	392.26	7.87	0.01	-187.58

TABLE II (continued)

¹Number of parameters included in model

² Log-likelihood

^a Plot distance is either field edge (5m from field edge) or field interior (55m from field edge)
^b Total bird species richness across all habitat types within each site used for all response variables except for crop dry mass, where bird species richness within cropland habitat was used because it provided a better fit to the data (see text for explanation of AICc modelling methods)

^c Treatment is exclosure vs. control plot

^d Rep is the timing of the repeated defoliation measurement, either during growth stage R1 or R5

4. AN ANALYSIS OF DIET IN A GENERALIST INSECTIVOROUS BIRD COMMUNITY: CLUES TOWARDS ECOSYSTEM SERVICE AND DISSERVICE PROVISION

4.1. <u>ABSTRACT</u>

DNA barcoding is an emerging technique that can be used detect prey from fecal samples, and often identify it to the species level. This is an especially useful technique when examining pest control services provided by birds, because species-level data allow us to determine whether birds are in fact consuming crop pests. Furthermore, we can use this information to determine if birds are also providing indirect disservices by eating "beneficial" arthropods such as predators that would otherwise consume pests. We used DNA barcoding to study the diets of communities of birds that inhabit the ecotone between soybean fields and grasslands, and likely forage in both habitats. We collected and sequenced DNA from 132 fecal samples from 25 bird species captured at six sites. We found that birds consumed an extremely large and varied diet which differed among both species and capture site. The large differences in diets suggest that birds were likely consuming prey opportunistically as available at each site. Of the nine most commonly detected prey species, three are known to be pests of soybeans. Bird diets also contained significantly more species of herbivorous prey than arthropods classified as natural enemies. Finally, we made the novel discovery that American Goldfinches, a highly granivorous species, consume arthropod prey, and may contribute to ecosystem service provision within this largely agricultural landscape. Together, our study shows that birds within this study system have the ability to consume a large variety of prey which suggests that they may be able to respond quickly to pest outbreaks and contribute to agricultural resiliency.

4.2. INTRODUCTION

Generalist insectivorous birds can provide valuable ecosystem services in agriculture by consuming arthropod crop pests (Sekercioglu et al. 2016). However, a growing body of literature suggests that these same birds can also provide indirect ecosystem disservices in agriculture when they consume "beneficial" prey such as arthropod natural enemies that would otherwise control pests (Martin et al. 2013, 2015; Garfinkel et al. 2020). The most direct way to predict whether a bird will provide services or disservices is by determining which arthropod species that bird consumes. By learning about differences in species- and site-specific diet preferences, we can begin to understand the factors that shift the balance of bird function between provision of net services versus disservices.

Ornithologists have historically studied bird diets using a variety of destructive or invasive methods. Most early studies relied on dissection of the stomachs of collected bird specimens (McAtee 1912). Other methods include the use of emetics to force regurgitation of stomach contents (Poulin and McNeil 1994, Diamond et al. 2007), and application of ligatures to nestlings to stop food from being swallowed (Rosenberg and Cooper 1990). These methods vary in their ability to distinguish arthropod species from each other, and prey items retrieved from stomachs are often too degraded to identify to species. More recently, ecologists have made use of high-throughput DNA sequencing technology to identify species via "DNA barcoding" (Valentini et al. 2009). Specifically, fecal samples are collected directly from birds without causing harm, and then DNA is extracted from the fecal sample. This resulting sample is a mixture of host, prey, and microbial DNA. Although DNA in fecal samples is degraded by digestion, use of specialized primers to amplify short "barcoding" regions allows detection of prey DNA, which can often be identified to the species level (Valentini et al. 2009). This is
especially useful when dealing with arthropods that can be identified to species only by visually examining small structures like genitalia. DNA barcoding, therefore, provides higher quality data in a less invasive way than traditional diet study methods. This makes it an ideal technique for examining potential pest control services and disservices by birds in agro-ecosystems, because species-level data are critical to assess whether birds actually consume important crop pest species.

While it is important to know about the diet of individual bird species, the whole bird community within a system may potentially contribute to net positive or negative effects on pest populations. Many previous studies have used DNA barcoding techniques to examine the diet of a single bird species (Jedlicka et al. 2017, McInnes et al. 2017, Sullins et al. 2018, McClenaghan et al. 2019). However, fewer have used DNA barcoding to examine bird diets at the wider community level (Crisol-Martínez et al. 2016). The advantage of comparing the diets of bird species within a community is that it provides information about how the community members contribute to net effects on the arthropod community (Maas et al. 2015).

In the Midwest region of the United States, many large-scale conventional agricultural fields are interspersed with small grasslands and prairies. Grasslands provide habitat for many bird species, and those birds forage for arthropods in the surrounding agricultural fields (Garfinkel et al. 2020). Soybeans (*Glycine max*) are one of the dominant crops in this region, and they are vulnerable to a variety of arthropod pest species (Hartman et al. 2015); the potential loss of soybean crop yield from arthropod pests is estimated to be approximately 11% (ranging from 4–16%) worldwide (Oerke 2006). Crop loss due to pests is expected to increase under a changing climate, with pest outbreaks potentially becoming more common (Walthall et al. 2012). With these expected increasing pressures, the ability to respond quickly to pest outbreaks will be

important to ensuring farmland resiliency. Specialist natural enemies, such as many parasitoids and other arthropod predators, require a constant food source even during times of low pest density in order to sustain their populations (Dosskey et al. 2017). Generalist insectivorous birds, on the other hand, are more adaptable to changing prey densities because they are highly mobile, and large enough to consume a wide variety of pest species. This implies that birds have the potential to provide valuable biological control services beyond those provided by arthropod natural enemies.

In this study, we used DNA barcoding to examine the diets of a community of birds within a mixed grassland-agricultural landscape in the Midwestern United States. Specifically, we studied the diets of birds captured at the ecotone between soybean fields and grasslands, which may forage in both habitats. The goals of our study were to 1) determine which arthropod species are consumed by birds within this mixed soybean/grassland system, and 2) examine differences in diet among bird species and sites. In particular, we compared the species richness of arthropod herbivores to arthropod natural enemies consumed by each bird, and examined bird consumption of known soybean pest species. Our ultimate objective is to provide data that may be useful in determining the ways that a bird community contributes to both ecosystem service and disservice provision.

4.3. METHODS

4.3.1. Study Sites

We collected fecal samples from birds for a DNA barcoding diet analysis at six sites in Kane, DeKalb, and Ogle counties in northern Illinois, USA (Figure 10). Because we were interested in the diets of birds that live and forage near both agricultural and grassland habitats, we selected sites that had a soybean field which shared at least one field edge with a grassland.

These grasslands were all owned by either county forest preserve districts or The Nature Conservancy and were managed in various ways to prevent forest encroachment and maintain native plant diversity (i.e. burning, mowing, and targeted control of invasive plant species). We only used sites where we were able to obtain permission from all involved public and private landowners (of both agricultural and grassland parcels) to conduct our research. Each capture site was separated from others by at least 1km. The average size of the soybean fields was approximately 34 ha, and the average size of the grassland was approximately 110 ha (see Table I, Appendix D for site identification, ownership, and measurements).



Figure 10. Six sites where birds were captured for fecal sample collection in northern Illinois, USA. Sites are represented by red stars, aFnd are all separated from each other by at least 1 km. All sites included a grassland directly adjacent to a soybean field, although the extent of grassland and cropland differed between sites. In the enlarged study site, the wavy light line is a

crushed limestone walking trail. 4.3.2. Fecal Sample Collection

We operated mist nets twice at each of our study sites, once in early June 2017 (from 1-11 June), and once in late June/July (from 29 June to 20 July). During each mist netting session, we set up nets at dawn and operated them until either 12 pm or until conditions became too hot and/or windy to continue. To capture birds that likely foraged in both habitat types, we placed the mist nets opportunistically between the soybean field and grassland, or within approximately 20 m of that edge within either habitat (except in one soybean field where we were able to access the field interior, and placed some nets approximately 50 m into the field interior). Because we could not remove or trim plants in the cropland or prairie, we placed the nets wherever the habitat provided a natural net lane where the net would not become entangled in vegetation. We operated between seven and ten mist nets (some 12m and others 9m in length) simultaneously during each banding session, with the goal of capturing as many birds as possible. Because the number of net hours at each site differed, we do not draw conclusions about bird abundance from our capture data.

We placed each bird extracted from the mist nets into a new brown paper bag for no more than 30 minutes (generally much less time) until it defecated. We used disposable gloves and spatulas, which were changed between birds, to avoid cross-contamination while collecting the fecal samples. We transferred each fecal sample to a labeled 2 ml tube with 90% ethyl alcohol (EtOH) and placed it on ice in an insulated cooler. We then banded the bird, collected standard measurements and demographic data, and released it. Once out of the field for the day, we stored the fecal samples at -20 °C.

4.3.3. DNA extraction and Sequencing

We extracted DNA from fecal samples using PowerSoil DNA Isolation Kits (Qiagen). During the bead-beating step of extraction, we homogenized our samples using a FastPrep-24 5G Homogenizer (MP Biomedicals). Library preparation, pooling, and sequencing were performed at the University of Illinois at Chicago Genome Research Core (GRC) within the Research Resources Center (RRC). Genomic DNA was PCR amplified with primers LCO1490F / COI-CFMRa (adapted from Jusino et al. 2019), which targets the COI gene of insectivorous animals. We selected these primers because they have been shown to amplify a higher percent of arthropod taxa from fecal samples than other commonly used barcoding primer pairs (Jusino et al. 2019). Amplicons were generated using a two-stage "targeted amplicon sequencing (TAS)" protocol as described in (Naqib et al. 2018). The primers contained 5' common sequence tags (common sequence 1 and 2, or CS1 and CS2, e.g. Moonsamy et al. 2013). Detailed PCR and sequencing methods are described in Appendix E.

4.3.4. **Bioinformatics and diet analysis**

We analyzed sequence data using an open-source bioinformatics pipeline, AMPTk, that has been optimized for handling amplicons of varying lengths, and which employs a variety of sequence quality filtration steps (Palmer et al. 2018). In short, AMPTk preprocesses the data by merging paired-end reads via USEARCH, and removes primers and trims sequences. It then clusters data into operational taxonomic units (OTUs) by employing a DADA2 de-noising algorithm followed by 97% clustering using VSEARCH. The sequences are then filtered to remove "index-bleed" between samples. Finally, OTUs are assigned taxonomy based on a hybrid taxonomy assignment method based on USEARCH/VSEARCH, UTAX, and SINTAX, and which makes use of the BOLDv4 database (Ratnasingham and Hebert 2007, Palmer et al. 2018).

We used the R package Phyloseq (McMurdie and Holmes 2014, R Core Team 2019) to further filter DNA sequence and taxonomic data. We began by filtering out all OTUs that were assigned to any phylum other than Arthropoda, or any class other than Insecta or Arachnida. Sequence read counts cannot be reliably used to infer the proportion of diet contributed by each OTU due to PCR biases (Jedlicka et al. 2017). We therefore transformed the OTU sequence read counts to OTU presence or absence for each sample. We first considered all OTUs with fewer than 10 reads per sample to be absent. We then normalized the sequence reads based on the total arthropod read counts per sample, and only considered OTUs with at least 0.01% of the reads to be present (see Deagle et al. 2019 for a discussion of varying methods to determine diet components from DNA barcoding). This allowed us to exclude low read-count OTUs that were most likely artifacts even if the total number of arthropod reads per sample was low. We used this transformed presence-absence dataset for all further statistical tests and diet summaries.

4.3.5. Arthropod Feeding Guild Determinations

We assigned a feeding guild to each detected arthropod species. We used Triplehorn and Johnson (2005) to assign guilds, and Parr et al. (2014) when needed. We broadly grouped arthropods into three guilds: natural enemy, herbivore, and "other". Natural enemies include predatory arthropods and parasitoids. Herbivores include arthropods that feed on living plant material in a way that can damage a plant (i.e. arthropods that consume pollen or nectar were not included in this category). The "other" category includes all species that do not fit into either of the previous categories, including detritivores and generalist omnivores. Arthropods that consume different food types at different life stages were placed into the "other" category only if they would be considered herbivores during one stage and natural enemies during another. Those

that would be grouped as natural enemy or herbivore during one life stage and "other" for a different life stage were grouped with either natural enemies or herbivores as appropriate.

4.3.6. <u>Diet Summaries and Statistical Analyses of Between-Group Differences in</u> Diet

For the analyses below, we examined three subsets of our bird community: 1) the entire bird community: data from all bird species sampled, 2) the community subset: data only from bird species represented by at least 5 fecal samples, and 3) data only from the Song Sparrow (*Melospiza melodia*), the bird species from which we collected the greatest number of fecal samples (Figure 11). Because we had an unbalanced design with varying numbers of fecal samples from each species and site, we used only summary statistics to describe the diets of the entire bird community. To statistically test for dietary differences between species and sites, we used the community subset data to ensure that all included bird species had sufficient replication (note that Crisol-Martínez et al. 2016 included bird species represented by at least 4 fecal samples in a similar analysis). Finally, we used the Song Sparrow subset to compare diets among sites within a single species.

For the community subset, we calculated the proportion of a fecal sample composed of herbivorous species by dividing the number of herbivore species per sample by the total arthropod species richness per sample. Although this value does not explain differences in total consumption of different insects (i.e. neither prey biomass nor abundance can be inferred), it does describe the diversity of species that have been consumed. We used non-parametric Kruskal-Wallis rank sum tests to determine whether the proportion of herbivores within a bird's diet differed by bird species or capture site. When Kruskal-Wallis tests indicated a significant difference between groups, we used Dunn's post-hoc test with Bonferroni adjustment to further

examine the between-group differences (Dunn 1964, Dinno 2017). We also used paired Wilcoxon signed rank tests to determine if fecal samples contained proportionally more herbivore species than natural enemies.

For both the community subset and the Song Sparrow data subsets, we used Sorenson distance matrices to compare the arthropod species that were present vs. absent in each fecal sample (i.e. comparing diet composition). We then used permutational multivariate analysis of variance (PERMANOVA) tests to determine whether there were significant differences in diet composition among bird species and among birds captured at different sites (for the Song Sparrow data subset, we only compared sites; Anderson 2017). PERMANOVA tests are generally less sensitive to heterogeneity in dispersions than other multivariate analysis methods, and so they often work well for ecological datasets (Anderson and Walsh 2013). Specifically, PERMANOVA tests the null hypothesis that the centroids of different groups in multivariate space are equivalent (Anderson and Walsh 2013). Therefore, a significant PERMANOVA test can be due to either differences in centroid location among groups, and/or differences in dispersion within groups. Consequently, when we found significant PERMANOVA test results, we followed up with a PERMDISP test, which specifically tests for heterogeneity of withingroup dispersion. In other words, a significant PERMANOVA test would indicate that diets differ among groups; a significant PERMDISP test would indicate that this was at least partly due to large variation in diets within groups.

We used the R package Vegan (Oksanen et al. 2019) to conduct PERMANOVA tests with 9999 permutations, and PERMDISP from the same package. We used Principal Coordinates Analysis (PCoA) plots to visualize differences in diet composition among species and among sites within the community subset data (Paliy and Shankar 2016). Within PCoA plots, we drew



ellipses around groups based on the assumption of a multivariate t-distribution (Wickham 2016).

Figure 11. Bird species included in DNA diet analysis. White numbers represent the number of fecal samples collected from each bird species. Bird species is indicated by the standardized 4-letter alpha code below each image, which are listed in Table I, Appendix F. Diet data were summarized at three levels (community, community subset, and the most frequently sampled species).

4.4. <u>RESULTS</u>

4.4.1. <u>Community Summary</u>

We collected and sequenced DNA from a total of 132 fecal samples from 25 bird species (Figure

11). Among the entire community DNA dataset, we clustered DNA sequences into 526

arthropod OTUs from 19 orders. Of those, we identified taxonomy to the species level for 326

arthropod species from 18 orders. We found a mean of 7.05 OTUs per sample (SD = 4.5, range =

0—22), and 6.5 OTUs identified to the species level per sample (SD = 5.2, range = 0—22). Six

fecal samples did not contain any arthropod species, and were therefore excluded from analyses

of diet guild (but not PERMANOVA analyses of between species and site differences, where they were retained as samples without any arthropods in their diet).

At the entire community level, four orders were detected in at least 50% of fecal samples: Diptera, Hymenoptera, Coleoptera, and Lepidoptera (Figure 12). Nine arthropod species were detected in at least 10 fecal samples each (Figure 13). Of these most commonly detected arthropod species, three are known to be potential pests of soybeans: *Calomycterus setarius* (Rice and Pilcher 1997); *Popillia japonica,* Japanese Beetle (Shanovich et al. 2019); and *Agrotis ipsilon,* Black Cutworm (Ogles et al. 2016). The most frequently detected species was *Calomycterus setarius,* the "imported long-horned weevil," which was present in 41.6% of fecal samples (Figure 13).



Figure 12. Percent of fecal samples containing DNA from arthropod orders in classes Arachnida and Insecta. N=132 fecal samples from 25 bird species.



Figure 13. Percent of fecal samples containing DNA from the most commonly detected arthropod species (i.e. detected in at least 10 fecal samples). Bars marked with an asterisk indicate that species is a known soybean pest. N=132 fecal samples from 25 bird species.

4.4.2. Community Subset

We collected at least 5 fecal samples from each of 7 bird species; these data were pooled into our "community subset" analyses (n=101 fecal samples, Figure 11). We included the American Goldfinch (*Spinus tristis*) in the community subset, although they are generally considered to be highly granivorous (McGraw and Middleton 2020). Including the American Goldfinch in these analyses provided a contrast to other more insectivorous species. Song Sparrows and Red-winged Blackbirds were the only bird species within the community subset to have consumed all three species of commonly detected soybean pest (Figure 14). Kruskal-Wallis tests indicated that the proportion of a diet composed of herbivorous species varied significantly by bird species (p = 0.035) but not by capture site (p = 0.203). However, post-hoc tests indicated this difference among species was due only to a significantly higher proportion of herbivorous species consumed by American Goldfinches than by Song Sparrows (p = 0.022; p > 0.05 for all other between-group comparisons). Paired Wilcoxon signed rank tests showed the proportion of diet composed of herbivorous arthropod species was significantly higher than the proportion composed of natural enemy species for the entire community subset (p < 0.001, Figure 15).

Although diet composition overlapped among both species and sites (Figure 16), PERMANOVA tests showed significant differences among bird species and sites, as well as a significant interaction between species and site (Table VI). PERMDISP tests showed significant heterogeneity of dispersion for bird species, and for the species × site interaction, but not for site (Table VI); this indicates that these differences were at least in part due to the heterogeneity of dispersion of diets among species and species × sites (i.e. diets within these groups varied). R^2 values indicated that species and species × site each explained more of the variation in diet than site alone (Table VI).

Table VI. Results of PERMANOVA test with 9999 permutations and PERMDISP tests on community subset diets.

Variable	df	R^2	р	PERMDISP <i>p</i>
Bird Species	6	0.13	0.0001	0.002
Site	5	0.06	0.0005	0.503
Bird Species:Site	11	0.11	0.0137	< 0.001
Residuals	78	0.69		



Figure 14. Percent of fecal samples containing DNA from soybean pests in seven bird species. Bird species is indicated by the standardized 4-letter alpha code below each image, which are listed in Table I, Appendix F.



Figure 15. Species richness (A) and proportion of diet (B) of natural enemies vs. herbivores per fecal sample in the community subset. Note that arthropods in diets were grouped by feeding guild into natural enemies, herbivores, or other, although the "other" category is not shown here. Therefore, the proportion of herbivores and the proportion of natural enemies within a fecal sample will not always sum to 1. Gray line is 1:1 reference. Points have been jittered to show detail. N=101 fecal samples.



Figure 16. PCoA plots of community subset bird diets grouped by A) bird species and B) capture site. Bird species in A) is indicated by the standardized 4-letter alpha code below each image, which are listed in Table 1, Appendix F. Sample locations in B) are described in Table I, Appendix D. Ellipses are drawn based on the assumption of a multivariate t-distribution. N=101 fecal samples.

4.4.3. Song Sparrow Diets

Our most frequently sampled bird species was the Song Sparrow, from which we obtained 40 fecal samples (Figure 11). We identified 128 arthropod species detected in fecal samples from Song Sparrows. The Song Sparrow was present at all six of our capture sites. Song Sparrow diet composition differed significantly between capture sites ($R^2 = 0.163$, p = 0.007). PERMDISP tests revealed significant heterogeneity of dispersion among sites (p < 0.001), which may partially reflect unbalanced sampling between sites. Most arthropod species (100 out of 128) were consumed by Song Sparrows at a single site (Figure 17); 12 arthropod species were detected in at least three of the six sites, and none were detected at all six sites.



Figure 17. Bipartite graph of arthropods identified to the species level detected in Song Sparrow (*Melospiza melodia*) diets. Each gray box at the base of the figure represents an arthropod species. Song Sparrows, represented by colored boxes at the top, were grouped by capture site. The width of the bar attached to the site boxes represents the number of Song Sparrows from that site which consumed the arthropod species to which the box is connected. The number of Song Sparrow fecal samples collected at each site is indicated in the top boxes.

4.5. DISCUSSION

This study demonstrates the advantages of a DNA barcoding approach to examining the diets and ecosystem service vs. disservice provision potential across a bird community. Our findings provide much higher species-level taxonomic data about bird diets in a grassland/agriculture system than have been previously described (Wiens and Rotenberry 1979). Our analysis of bird diets across multiple scales (the community, community subset, and Song Sparrows) suggests that these generalist insectivorous birds in a mixed agriculture/grassland system have surprisingly large and varied diets, with a total of 326 arthropod species detected across all samples.

Diet composition of the community subset differed significantly among species and among sites. However, differences among species were contingent upon site, and similarly, differences among sites were contingent upon species (Table VI). This significant interaction indicates that differences in bird diets among species varied by capture site. Song Sparrow diets varied significantly among sites, with the majority of prey species detected at only a single site (Figure 17). Although we do not have data describing the available arthropod communities at each site, these results may suggest that birds were exploiting food resources opportunistically based on availability. This is consistent with the findings of an earlier study on grassland bird diets (Wiens and Rotenberry 1979). While stomach-sampling methods such as those used in Wiens and Rotenberry (1979) can provide information on dietary composition (i.e. the relative biomass of various prey items), they do not give an accurate estimation of species richness of consumed prey. Our data, on the other hand, emphasize the large species-level diversity of prey items consumed by birds in our study system.

The ability of birds to consume a large variety of prey species, as well as the ability to

exploit food resources opportunistically, has important implications for ecosystem service provision by birds. These data suggest that birds should be able to respond very quickly to pest outbreaks or increased pest densities (Whelan et al. 2008). In fact, the frequent consumption of the Japanese Beetle (*Popillia japonica*) within the bird community supports this (Figure 13 and Figure 14). Japanese beetles can cause economically important damage to a variety of crops, including soybeans (Shanovich et al. 2019). We conducted a smaller scale DNA barcoding diet study in a similar agroecosystem in the year prior to this one (Garfinkel et al. 2020), and did not find any evidence of consumption of Japanese beetles (although note that we used different DNA primers for the previous study, so those data are not perfectly comparable). As part of the annual Illinois corn and soybean survey, Japanese beetles were found in much higher densities during the year of our study than the previous year (Estes 2017). The widespread consumption of Japanese beetles we report in this study therefore likely represents opportunistic foraging on an increasingly common prey species.

While the birds in our study system show potential to respond to pest outbreaks, there is also evidence that they can provide indirect disservices by consuming arthropod natural enemies (see previous chapter). However, we determined that the community subset birds consumed significantly more herbivorous arthropod species than natural enemies (Figure 15). This could reflect opportunistic foraging if herbivorous species were more common than natural enemy species, or alternatively, selective foraging on the herbivore prey species. In order to disentangle opportunistic vs. selective foraging behaviors, we would need to know more about prey availability at each site. To further quantify the relative contribution of each bird to service or disservice provision, we would also need to determine the proportional contribution of each arthropod species to the overall diet (which cannot be determined with current DNA barcoding

technology; (Jedlicka et al. 2017). This is an important limitation of our study, and as DNA barcoding technology advances, hopefully methodologies will be developed to overcome this limitation in future studies. The ability to compare the number of individual natural enemies consumed to the number of herbivores would allow use to predict more exactly whether certain bird species are likely to provide net services vs. disservices.

We found that two bird species from the community subset consumed all three species of soybean pests: Song Sparrows and Red-winged Blackbirds (Figure 14). These two species may exert disproportionately large effects within our study system, because they are both widespread species that can be found at higher densities than more typical grassland species (see point count data from the previous chapter, which was conducted at the same sites as this study). Red-winged Blackbirds have historically been blamed for damage to grain crops such as rice and corn, although research suggests they likely do not decrease crop yield as much as farmers perceive them to (Weatherhead et al. 1982, Borkhataria et al. 2012). Our findings suggest, in fact, that Red-winged Blackbirds along with Song Sparrows may provide an important but overlooked service within this study system.

Another bird species of interest that we examined within our community subset was the American Goldfinch (*Spinus tristis*). Although they are a highly granivorous species (McGraw and Middleton 2020), we included them in the study as a comparison to more insectivorous species, and because they were common in all of our study sites (see previous chapter). Indeed, the PCoA analysis shows the American Goldfinch diet overlaps with other species, but is nevertheless more distinct than other species (Figure 16A). It is of note that we detected arthropod DNA in fecal samples from goldfinches despite their extremely granivorous nature. This may be due to the fact that the DNA metabarcoding approach cannot distinguish between

arthropod life stages that have been consumed. Arthropods consumed as adults, larvae, or eggs should all appear as presences in the diet composition data. It is possible, therefore, that the goldfinches consumed tiny nymphs, larvae, or eggs on plant material while foraging for seeds. In particular, the most commonly detected arthropod in goldfinch samples was *Larinus planus*, a species of weevil that lays eggs in thistle heads (Havens et al. 2012). Thistle seeds are also a preferred food of goldfinches (McGraw and Middleton 2020), and this diet overlap could lead to unintended weevil consumption by goldfinches. In addition, although we limited our dataset to species that were in the phylum Arthropoda and either class Insecta or Arachnida, we did not make further judgements on whether species were intentionally consumed as prey or otherwise. Therefore, our dataset includes arthropods such as fleas and mites that may have been accidentally or inadvertently consumed during preening behaviors.

We found that American Goldfinches consumed a higher proportion of herbivorous species than Song Sparrows, and we detected DNA from two soybean pests in goldfinch diets (Figure 14). Even if goldfinches consumed only pest arthropod eggs or larvae, this represents a previously undescribed contribution to pest control by a largely granivorous species. We recommend that future study should further examine this phenomenon to confirm whether goldfinches are indeed consuming eggs rather than adult pests. If this is the case, it would be important to further determine whether they consume them in high enough quantities to substantially affect pest populations.

While we were unable to determine that any one bird species or capture site had a higher probability of providing services than disservices, we did identify the American Goldfinch as a species that potentially contributes to ecosystem service provision more than previously expected based on the assumption that it is an obligate granivore.

4.5.1. <u>Conclusions</u>

The combined results of our study of bird diets within a mixed agriculture/grassland system have several implications for predicting net service vs. disservice provision by birds. First, we show that these birds have an extremely varied diet, and likely respond opportunistically to prey availability. This supports the theory that birds can contribute to agricultural resiliency against pest outbreaks. Second, we show that the bird community consumed more species of herbivorous arthropods than natural enemies. In addition, three of the nine most commonly consumed arthropods in the bird community were known pests of soybeans. When herbivore density is high enough to decrease crop yield, birds may provide net services, although when herbivore biomass is low compared to natural enemies, we may see net disservices by birds. Finally, we identify two bird species, Red-winged Blackbirds and Song Sparrows, that are both common and consumed all three soybean pest species, and a third, the American Goldfinch, which potentially contributes to arthropod pest control despite the previous understanding that it is highly granivorous. Future research should further examine the ability of these bird species to contribute to pest-removal services in soybean agriculture. Together, these results enhance our current knowledge of trophic effects exerted by a bird community which may have economic consequences in agriculture.

4.6. ACKNOWLEDGEMENTS

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4.8. <u>APPENDICES</u>

4.8.1. <u>Appendix D Table I:</u> Approximate area in hectares of the soybean field and grassland patches at six study sites, Illinois, USA.

Site ¹	Ownership of Grassland	Area Grassland (Ha)	Area Soybeans (Ha)
DY	Kane County Forest Preserve District	240	4
РО	Dekalb Country Forest Preserve District	11	83
MS	Kane Country Forest Preserve District	175	35
FL	The Nature Conservancy	94	10
SB	The Nature Conservancy	45	27
WH	The Nature Conservancy	94	44

¹DY = Dick Young Forest Preserve; PO=Prairie Oaks Forest Preserve; MS = Muirhead Springs Forest Preserve; FL = Nachusa Grasslands at Flagg and Lowden roads; SB = Nachusa Grasslands at Stone Barn Road; WH = Nachusa Grasslands at White House

4.8.2. Appendix E: PCR conditions and technical sequencing methods

We used a forward primer with the sequence: <u>ACACTGACGACATGGTTCTACA</u> GGTCAACAAATCATAAAGATATTGG (linker portion is underlined) and a reverse primer with the sequence <u>TACGGTAGCAGAGACTTGGTCT</u>GGWACTAATCAATTTCCAAATCC (Jusino et al. 2019). First stage PCR amplifications were performed in 10 microliter reactions in 96-well plates, using MyTaq HS 2X mastermix (Bioline). PCR conditions were 5 cycles of: 95°C for 60 s, 45°C for 90 s, 72°C for 90 s; 28 cycles of: 94°C for 60 s, 50°C for 90 s, 72°C for 60 s. Subsequently, a second PCR amplification was performed in 10 microliter reactions in 96well plates. A mastermix for the entire plate was made with MyTaq HS 2X mastermix. Each well received a separate primer pair with a unique 10-base barcode, which was obtained from the Access Array Barcode Library for Illumina (Fluidigm, South San Francisco, CA; Item# 1004876). These primers contained the CS1 and CS2 linkers at the 3' ends of the oligonucleotides. Cycling conditions were: 95°C for 5 minutes, followed by 8 cycles of 95°C for 30", 60°C for 30" and 72°C for 30". Samples were subsequently pooled in equal volume using an EpMotion5075 liquid handling robot (Eppendorf, Hamburg, Germany). The pooled library was purified to remove fragments smaller than 200 bp using an AMPure XP cleanup protocol (1X, vol/vol; Agencourt, Beckmann-Coulter). The pooled libraries, with a 20% phiX spike-in, was loaded onto an Illumina MiniSeq mid-output flow cell (2x153 paired-end reads). The amplicons (before purification) were-pooled based on the distribution of reads per barcode, to generate a more balanced distribution of reads. The re-pooled library was purified using AMPure XP cleanup, as described above. The re-pooled libraries, with a 20% phiX spike-in, were loaded onto a MiniSeq flow cell, and sequenced (2x153 paired-end reads). Fluidigm sequencing primers (targeting the CS1 and CS2 linker regions) were used to initiate sequencing. De-multiplexing of reads was performed on instrument.

Common Name	Scientific Name	Alpha Code
American Goldfinch	Spinus tristis	AMGO
American Robin	Turdus migratorius	AMRO
Baltimore Oriole	Icterus galbula	BAOR
Barn Swallow	Hirundo rustica	BARS
Black-capped Chickadee	Poecile atricapillus	BCCH
Bobolink	Dolichonyx oryzivorus	BOBO
Brown Thrasher	Toxostoma rufum	BRTH
Cedar Waxwing	Bombycilla cedrorum	CEDW
Chipping Sparrow	Spizella passerina	CHSP
Common Yellowthroat	Geothlypis trichas	COYE
Dickcissel	Spiza americana	DICK
Eastern Meadowlark	Sturnella magna	EAME
Eastern Phoebe	Sayornis phoebe	EAPH
Field Sparrow	Spizella pusilla	FISP
Grasshopper Sparrow	Ammodramus savannarum	GRSP
Henslow's Sparrow	Ammodramus henslowii	HESP
House Wren	Troglodytes aedon	HOWR
Indigo Bunting	Passerina cyanea	INBU
Orchard Oriole	Icterus spurius	OROR
Red-winged Blackbird	Agelaius phoeniceus	RWBL
Savannah Sparrow	Passerculus sandwichensis	SAVS
Sedge Wren	Cistothorus platensis	SEWR
Song Sparrow	Melospiza melodia	SOSP
Vesper Sparrow	Pooecetes gramineus	VESP
Willow Flycatcher	Empidonax traillii	WIFL

4.8.3. <u>Appendix F Table I:</u> Standardized alpha codes for the bird species sampled in this study. Species are listed in alphabetic order of common names.

GENERAL APPENDICES

Appendix G: License for use of previously published chapter ("Birds suppress pests in corn but release them in soybean crops within a mixed prairie/agriculture system") in this dissertation.

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Appendix G continued

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Office of Animal Care and Institutional Biosafety Committee (OACIB) (M/C 672) Office of the Vice Chancellor for Research 206 Administrative Office Building 1737 West Polk Street Chicago, Illinois 60612

3/15/2018

Christopher J. Whelan Biological Sciences M/C 066

Dear Dr. Whelan:

The protocol indicated below was reviewed in accordance with the Animal Care Policies and Procedures of the University of Illinois at Chicago and **renewed on 3/15/2018.**

Title of Application:Quantifying services and disservices provided by birds in conventional agricultureACC NO:16-043Original Protocol Approval:5/9/2016 (3 year approval with annual continuation required).Current Approval Period:3/15/2019

Currently protocol NOT matched to specific funding source. Modification will need to be submitted prior to Just in time or acceptance of award to match protocol to external funding source. All animal work proposed in the funding application must be covered by an approved protocol.

UIC is the only performance site approved under this protocol.

This institution has Animal Welfare Assurance Number A3460.01 on file with the Office of Laboratory Animal Welfare, NIH. This letter may only be provided as proof of IACUC approval for those specific funding sources listed above in which all portions of the grant are matched to this ACC protocol.

Thank you for complying with the Animal Care Policies and Procedures of the UIC.

Sincerely,

Timothy J. Koh, PhD Chair, Animal Care Committee TJK/kg cc: BRL, ACC File, Emily Minor, Megan Garfinkel

Phone (312) 996-1972 • Fax (312) 996-9088

MEGAN B. GARFINKEL

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PROFESSIONAL PREPARATION		
Institution University of Illinois at Chicago (4.0 GPA) <i>Chicago, IL</i>	Major Biology	Degree and Year PhD Expected May 2020
Humboldt State University (4.0) Arcata, CA	Wildlife Biology	M.S. 2013
Skidmore College (3.79) Saratoga Springs, NY	Biology	B.A. 2008

SELECTED APPOINTMENTS

2015-present	Teaching Assistant, Department of Biological Sciences, UIC
2010, 2013-14	Ornithologist, Starr Ranch, Audubon CA
2012-2013	Graduate Assistant, Department of Wildlife, HSU
2011-2013	Wright Refuge Banding Station Manager, HSU
2010-2011	Head LTREB (NSF) research technician, Smithsonian Migratory Bird Ctr.

PEER-REVIEWED PUBLICATIONS

- 1. **Garfinkel, M**., Minor, E. S., and Whelan, C. J. 2020. Birds suppress pests in corn but release them in soybean crops within a mixed prairie/agriculture system. *The Condor*: duaa009.
- Minor, E.S., Anderson, E.C., Belaire, A.J., Garfinkel, M., and Smith, A.D. 2017. Urban green infrastructures and ecological networks for urban biodiversity conservation In Ossola, A. and Niemelä, J. (Eds.), Urban Biodiversity: from research to practice (pp. 186-199). London: Routledge.
- 3. Milligan, M., Johnson, M. D., **Garfinkel, M.**, Smith, C., and Njoroge, P. 2016. Quantifying pest control services by birds and ants in Kenyan coffee farms. *Biological Conservation* 194: 58-65.
- 4. **Garfinkel, M.**, and Johnson, M. D. 2015. Pest-removal services provided by birds on small organic farms in northern California. *Agriculture, Ecosystems and Environment* 211: 24-31.
- 5. Mettke-Hofmann, C., Hamel, P., Hofmann, G., Pellegrini, A., Mizen, J., **Garfinkel, M.**, Zenzal, T., Huston, E., Schiff, N., and Greenberg, R. 2015. Competition and habitat quality influences age and sex distribution in wintering Rusty Blackbirds. *PLOS One* 10, e0123775.
- 6. Cooper, N., Thomas, M., **Garfinkel, M.**, Schneider, K., and Marra, P. 2012. Comparing the precision, accuracy, and efficiency of branch clipping and sweep netting for sampling arthropods in two Jamaican forest types. *Journal of Field Ornithology* 83(4): 381-390.

- Gordon, W., Roy, M., Vardar-Ulu, D., Garfinkel, M., Mansour, M. Aster, J., and Blacklow, S. 2009. Structure of the Notch1 Negative Regulatory Region: Implications for Normal Activation and Pathogenic Signaling in T-ALL. *Blood* 113(18): 4381-90.
- Tang, Y., Poustovoitov, M., Zhao, K., Garfinkel, M., Canutescu, A., Dunbrack, R., Adams, P., and Marmorstein, R. 2006. Structure of a human ASF1a–HIRA complex and insights into specificity of histone chaperone complex assembly. *Nature Structural and Molecular Biology* 13: 912-929.

GRANTS and AWARDS

- Department of Biological Sciences Graduate Teaching Award. May 2019.
- *Prairie birds in agriculture: Examining the use of surrounding agricultural habitat by birds that live in prairies.* 2017 and 2018. Friends of Nachusa Grasslands Scientific Research Project Grant. \$2000 (2017), \$3000 (2018).
- *Examining bird-mediated trophic interactions within a mixed agriculture and prairie landscape*. 2017 and 2018. UIC Department of Biological Sciences Hadley Grant. \$3000 (2017), \$4000 (2018).
- Bird-provisioned pest removal services on conventional farms: an evaluation of an ecosystem services approach as a tool for prairie bird conservation, 2016-2017. Institute for Environmental Science and Policy Predoctoral Fellowship. \$10,000.
- Annie's Homegrown Sustainable Agriculture Scholarship, 2016. \$11,000.
- Bird-provisioned pest removal services on conventional soy farms: an evaluation of an ecosystem services approach as a tool for prairie bird conservation, 2016-2017, American Ornithologist's Union Research Award. \$2000.
- *Quantifying Services And Disservices By Birds In Soy Agriculture*, 2016-2017, UIC Chancellor's Graduate Research Award. \$8,000.
- Pest-removal services provided by songbirds on small organic farms in Humboldt County, CA, 2011-2013, Wright Refuge Grant/Fellowship. \$60,810 including funding for research, stipend, and tuition.

SELECTED POSTERS and PRESENTATIONS

• Invited Speaker to present August 2020. Bird trophic effects on corn and soybean crops within a mixed grassland and agricultural landscape. Lightning talk in symposium entitled "Protecting and restoring bird habitat in the agricultural matrix: net benefits for birds and farmers." To be presented at North American Ornithological Conference (NAOC) virtual meeting.

SELECTED POSTERS and PRESENTATIONS (Continued)

- August 2019. Pest control services and disservices provided by birds in corn and soybean agriculture. Poster presented at Ecology Society of America's annual conference, New Orleans, LA.
- Invited Speaker. February 2019. The benefits of birds on farms. Oral presentation to Will County Audubon monthly meeting, Joliet, IL.
- Invited Speaker. October 2017. Do prairie birds provide services or disservices on adjacent conventional farms? Oral presentation at Friends of Nachusa Science Symposium, Franklin Grove, IL.
- Invited Speaker. August 2017. Can birds provide ecosystem services in North American industrial agriculture? Symposium oral presentation at the American Ornithological Society Conference, East Lansing, MI.
- October 2016. Bird provisioned pest removal services on conventional farms: an evaluation of an ecosystem services approach as a tool for prairie bird conservation. Poster presented at Friends of Nachusa Science Symposium, Franklin Grove, IL.
- **Invited Speaker.** August 2016. Future directions for bird-provisioned pest control studies in conventional agricultural systems. Symposium oral presentation at North American Ornithological Conference, Washington D.C.
- September 2014. Pest-removal services provided by songbirds on small organic row crop farms in Humboldt County, CA. Oral presentation at AOU/COS/SCO Joint Meeting, Estes Park CO

SCIENTIFIC OUTREACH

I have been presenting bird banding demonstrations and classes to the public for over 10 years. I have performed demonstrations to children from grades K-12, undergraduate and graduate students, and adults. I have taught over 70 individuals how to handle and band wild birds. I have also mentored and provided research experience for 15 UIC undergraduate students, many of whom come from backgrounds that are under-represented in the STEM fields.