LADYBIRDS: CONSERVATION, ECOLOGY AND INTERACTIONS WITH OTHER ORGANISMS

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LADYBIRDS: CONSERVATION, ECOLOGY AND INTERACTIONS WITH OTHER ORGANISMS

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Editorial: Ladybirds: Conservation, ecology and interactions with other organisms

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Editorial on the Research Topic
Ladybirds: Conservation, ecology and interactions with other organisms

Human impact on the natural world has expanded enormously over the past century and a half, with rapid population growth and an increasingly globalized economy causing pollution, habitat destruction, and climate change (Johnson et al., 2017). This threatens biodiversity and proper functioning of natural ecosystems, which we depend on for resources (wood, food, etc.) and ecological services (water storage and purification, waste treatment, pollination, etc.) (Vilà et al., 2006; Newbold et al., 2015; Dasgupta, 2021). One major problem is the ecological disturbance caused by organisms invading ecosystems outside their natural ranges—these invasions may occur inadvertently, for example through international trade, or be the result of deliberate introductions that have got out of control (e.g., Allendorf and Lundquist, 2003; Dunn and Hatcher, 2015; Pfliegler et al., 2018). Invading species disrupt the normal functioning of ecosystems by directly outcompeting native species, destabilizing food webs, or affecting the cycling of nutrients through the ecosystem. Almost without exception, invading species set in motion a chain of ecological effects that eventually result in ecosystems that are less healthy and less productive. Invasive alien species (IAS) are often considered pests and can drive biodiversity declines globally (Mollot et al., 2017). A prime example of one such deliberate introduction that has gone awry is that of *Harmonia axyridis*, the harlequin ladybird (Brown et al., 2011; Roy et al., 2016).

Ladybirds (Coleoptera, Coccinellidae) are primarily predaceous insects. They play a crucial role in the regulation of herbivorous insect populations in natural and managed systems. For decades, a countless number of individuals of different species have been introduced in regions to which they were not native as part of classical biological control strategies. However, from a conservationist viewpoint, these approaches have been revealed to have negative effects on native biota (Soares et al., in press; Rondoni et al., 2021). In the last 30 years, populations of many formerly common native species of ladybird have declined in several countries in North and South America and Europe. This occurred in tandem with increasing populations of two IAS, *Coccinella septempunctata* (seven-spotted ladybird) in North America and *H. axyridis* globally (e.g., Evans et al., 2011; Roy et al., 2016; Brown and Roy, 2018; Camacho-Cervantes et al., 2017; Hiller and Haelewaters, 2019).

Ladybirds face many threats. Some species might be at risk of extinction due to habitat loss and fragmentation, invasive species, pollution by agrochemicals, climate change, and overexploitation of resources (e.g., Harvey et al., 2020). In light of this, the International Union for Conservation of Nature (IUCN) Species Survival Commission (SSC) Ladybird Specialist Group consisting of 50 members was recently established, with the following goals: (*i*) identify ladybird species with high extinction risk, (*ii*) determine the factors leading to high extinction risk, (*iii*) develop strategies to manage risk and improve the conservation status of these species, and (*iv*) implement conservation management for threatened ladybird species (IUCN SSC Ladybird Specialist Group, 2018). As a first major output from this group, Soares et al. (in press) reviewed the major ecological threats currently facing ladybirds and identified actions contributing to the conservation and recovery of ladybird populations. These authors proposed a roadmap for ladybird conservation strategies at different timescales. Conservation of locally native ladybirds does not only depend on the collation of existing information (for IUCN Red List assessments), but also on active collaborations among stakeholders (researchers, citizen scientists, conservation practitioners) to share data, coordinate standardized surveying efforts, and efficiently disseminate results.

The eleven papers in this Research Topic contribute directly to this conservation effort, through actions and solutions at short-term, mid-term, and long-term timescales (Soares et al., in press). Three papers address short-term, so-called “no-regret” solutions towards education for awareness, citizen science, and capacity building. The European Ladybirds smartphone application (Skuhrovec et al.) is a collaborative citizen science project to compile records of ladybirds across Europe and assess distributional changes over time, while connecting and engaging people in nature and increasing awareness about the diversity and ecological importance of ladybirds. Weyman et al. evaluate the role of citizen scientists in collecting distribution data in Ireland based on the submission of pictures through social media channels, using ladybirds as a case example. Stowe et al. demonstrate that pollen and nectar increase reproductive success and alter oviposition patterns in *Hippodamia convergens*, highlighting the importance of floral resources in the agricultural landscape to the conservation of this aphid predator and its biocontrol services.

Several papers address mid-term actions. Zakharov et al. present new records of *H. axyridis* in Eastern Europe, showing occupation of new territory from the western borders of Russia to the Volga River, mainly in 2018–2020 and with a dispersal rate of 200 km/year. Two studies discuss the species composition and abundance of ladybirds in the center and outskirts of Prague, Czechia. Honek et al. reveal that the warm urban mesoclimate significantly supports the seasonal dynamics of IAS *H. axyridis*, whereas Skuhrovec et al. show that species richness and abundance of native ladybirds are lower in the urban center compared to the outskirts. Farrow et al. explore why *Coccinella quinquepunctata* is restricted to exposed riverine sediments in Wales and Scotland. The authors rule out an effect exerted by *H. axyridis*, but, instead, highlight negative impacts by an invasive alien herbaceous plant, *Impatiens glandulifera*: its shallow roots lead to increased susceptibility to erosion, and its effect on soil microbial communities results in homogenization of the native plant community. In their second contribution, Farrow et al. present the results of their standardized sampling in rural woodland sites in the UK and find that (1) *H. axyridis* does not dominate ladybird communities in these habitats and (2) there are distinct native ladybird communities associated with woodland type.

Systematic long-term monitoring of ladybirds, based on standardized sampling protocols, is a fundamental tool to understand species population dynamics. The study by Hesler and Beckendorf summarizes results from 14 years of sampling ladybirds in five field crops and restored prairie in eastern South Dakota, USA. Sample rates of ladybirds in alfalfa, spring grains, and corn are decreased compared to those of a previous long-term survey. Native adult ladybirds show a significant declining trend in corn but not in other habitats. The authors discuss their findings in light of prey availability in various crops and landscape variables.

Two papers of the Research Topic focus on natural enemies of *H. axyridis*. de Groot and Haelewaters review what is known about simultaneous infections of *H. axyridis*, highlighting the importance of incorporating multiple species interactions in future studies of this ladybird. Finally, Haelewaters et al. evaluate the effects of selected host, climate, and landscape variables on infection probability of *H. axyridis* with the microfungal ectoparasite *Hesperomyces harmoniae* (Haelewaters et al., in press). This community ecology work may prove vital for integrated pest management.

Author contributions

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Declining Abundance of Coccinellidae (Coleoptera) Among Crop and Prairie Habitats of Eastern South Dakota, USA

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Lady (= ladybird) beetles (Coleoptera: Coccinellidae) provide agroecosystem services as major predators of aphids and other pests of field crops. Several native coccinellids in North America have declined in association with the introduction of invasive species of lady beetles. In particular, populations of three native species declined drastically (*Coccinella transversoguttata richardsoni*) or effectively disappeared (*Coccinella novemnotata*, *Adalia bipunctata*) from agricultural landscapes in eastern South Dakota, U.S.A., following establishment of an invasive coccinellid (*Coccinella septempunctata*) in the 1980s. Since then, two other non-native coccinellids (*Harmonia axyridis* and *Hippodamia variegata*) have established in eastern South Dakota, but long-term analysis of their impact on the aphidophagous coccinellid guild is lacking. This paper summarizes long-term results from 14 years (2007–2020) of sampling coccinellids by sweepnet and timed searches in five field crops and restored prairie in eastern South Dakota. In all, 17,338 aphidophagous coccinellids comprising 10 species were sampled. Two invasive species (*Coc. septempunctata*, *Har. axyridis*) were the third- and fourth-most abundant species, respectively. The seven most abundant species constituted 99% of all coccinellids sampled and were recorded from all six habitats. However, coccinellid species ranged considerably in their evenness of habitat use, resulting in differences in rank abundance among habitats. Coccinellid assemblages were similar for alfalfa and winter wheat, but not for other habitats, which possessed distinct coccinellid assemblages based on rank abundance. Annual abundance of coccinellids varied considerably within habitats, but declining trends were evident from significant negative regressions in annual abundance for adult and immature coccinellids in corn and adults in soybean. As a group, native adult coccinellids showed a significant declining trend in corn but not in other habitats, whereas trends for non-native adult coccinellids were non-significant in all habitats. Sample rates of coccinellids in alfalfa, spring grains, and corn in this study were 74, 26, and 6%, respectively, compared to that of a previous study from the region, further indicating substantial decreases in coccinellid abundance. Possible explanations and implications for observed patterns in coccinellid diversity and individual species abundances in field crops and restored prairie of eastern South Dakota are discussed with respect to prey, agronomic trends, and landscape factors.

Keywords: beetle conservation, invasive species, habitat use, biodiversity, lady beetles

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INTRODUCTION

Eastern South Dakota, USA, was historically part of the tallgrass prairie portion of the North American Great Plains before conversion of a large majority of land to agriculture in the mid-1800s (Maizel et al., 1998). Less than 14% of tallgrass prairie remains in North America (Samson et al., 2004). In its place, field crops—mainly corn, soybeans, alfalfa, and small grains such as wheat and oats—have become a major component of the landscape (Dumke and Dobbs, 1999; Rashford et al., 2011; Wright and Wimberly, 2013).

Each of the field crops in eastern South Dakota is colonized annually by its own complex of arthropod pests that includes aphids, defoliators, and stem borers (Bing et al., 1999; Hesler et al., 2000, 2005, 2018; Hutchinson et al., 2010; Lundgren et al., 2013; **Supplementary Table 1**). In turn, these pests have a complex of natural enemies, including a guild of predacious lady (=ladybird) beetles, or coccinellids (Elliott and Kieckhefer, 1990a; Elliott et al., 1996; Lundgren et al., 2013; Hesler, 2014). Coccinellids contribute substantially to pest suppression, with both adults and larvae preying upon pests (Obrycki and Kring, 1998; Michaud, 2012). Thus, their conservation is vital to sustainable agriculture, and long-term studies on their effectiveness and preservation are important (Obrycki and Kring, 1998; Iperti, 1999; Honek et al., 2014).

Extensive surveys of coccinellids of east-central South Dakota commenced in the 1970s in small grains, alfalfa, and corn (Kieckhefer et al., 1992). Six species—*Hippodamia convergens, Hippodamia parenthesis*, *Hippodamia tredecimpunctata tibialis*, *Coleomegilla maculata lengi, Coccinella transversoguttata richardsoni*, and *Cycloneda munda*—were common to all three crops, whereas *Adalia bipunctata* was only sampled from corn. *Coccinella novemnotata*, a species of current conservation concern (Harmon et al., 2007), was only sampled in low numbers on adhesive traps adjacent to fields.

However, coccinellid assemblages of eastern South Dakota cropland have been subjected to various changes since the Kieckhefer et al. (1992) survey. These changes include establishment of new coccinellid species, introduction of an invasive pest, and changes in vegetational composition of the landscape. For instance, three non-native coccinellids have been detected in east-central South Dakota. The first species, *Coccinella septempunctata*, established in the region in 1987 and quickly became prevalent in alfalfa, spring grains, and corn (Elliott et al., 1996). Moreover, its establishment was associated with significant change in native coccinellid community structure that related to reduced abundances of *A. bipunctata* and *Coc. transversoguttata richardsoni* in those crops (Elliott et al., 1996). Two other non-native coccinellids, *Harmonia axyridis* and *Hippodamia variegata*, were detected in eastern South Dakota in 1996 (Hesler et al., 2001) and in 2010 (Hesler and Lundgren, 2011), respectively, but long-term analysis of their impact on coccinellid assemblages is lacking. Both species are generalists that compete with native coccinellids in various habitats (Koch, 2003; Gardiner et al., 2011). *Harmonia axyridis* is considered invasive and has often become dominant in coccinellid assemblages in North America, whereas *Hip. variegata* establishes relatively low abundance (Koch, 2003; Lucas et al., 2007; Gardiner et al., 2009; Lamb et al., 2019). *Coccinella septempunctata* has typically remained relatively abundant in various North American habitats following the addition of other invasive coccinellids (Brown, 2003; Alyohkin and Sewell, 2004; Lucas et al., 2007; Gardiner et al., 2009).

Before 2000, soybeans in South Dakota and other parts of the northern Great Plains were characterized by a set of arthropod pests that caused only sporadic economic damage (Lambert and Tyler, 1999; Ragsdale et al., 2011). Consequently, field studies of coccinellids in eastern South Dakota deliberately omitted soybean due to its lack of pests (Kieckhefer et al., 1992; Hesler et al., 2000). However, in 2000, establishment of an invasive pest, the soybean aphid (*Aphis glycines*), greatly increased the risk of economic injury to soybean and led to enormous increases in insecticide application in northern production areas (Ragsdale et al., 2011). Despite widespread insecticide use against soybean aphid, a large complex of aphid natural enemies, including both native and non-native coccinellids, became associated with soybean (Schmidt et al., 2008; Gardiner et al., 2009; Lundgren et al., 2013; Hesler, 2014).

Finally, regional cropping patterns changed substantially over the last 40 years. Notably, plantings of corn and soybean continue to steadily increase at the expense of spring grains and winter wheat and by additional conversion of grassland and wetland [Johnston, 2013; Wright and Wimberly, 2013; NASS (National Agricultural Statistics Service), 2021]. Such changes in land use may impact coccinellid assemblages and influence biological control of insect pests in cropping systems (Bianchi et al., 2007; Landis et al., 2008; Gardiner et al., 2009; Cox et al., 2014). In particular, increasing dominance by a crop such as corn simplifies agricultural landscape diversity, which reduces the supply of natural enemies available in an area (Landis et al., 2008).

Consequently, only limited amounts of tallgrass prairie have been restored (Samson et al., 2004). Some researchers have hypothesized that tallgrass prairie and other natural areas may serve as alternative, refuge habitats for coccinellids (Hesler and Petersen, 2008; Diepenbrock and Finke, 2013). To date, relatively modest numbers of coccinellids have been documented in tallgrass prairie (Hesler et al., 2005; Hesler and Petersen, 2008; Diepenbrock and Finke, 2013), but further comparison of their utilization by coccinellids vs. field-crop habitats is warranted.

New studies on coccinellid assemblages in eastern South Dakota are sorely needed in light of these major changes in the agricultural landscape. Long-term sampling conducted at the same location is necessary to reliably delineate trends in fauna that may change relatively slowly but exhibit high temporal variability (Elliott et al., 1996; Strayer et al., 2006; Honek et al., 2016). In particular, we would expect long-term monitoring of coccinellids in eastern South Dakota to establish the following outcomes, namely that (1) *Coc. septempunctata* would maintain a relatively moderate to high abundance, (2) *Har. axyridis* would become one of the dominant species in coccinellid assemblages, (3) *Hip. variegata* would establish low (i.e., 1–5%) relative abundance, (4) at least one native coccinellid would show a significant decline in abundance over the course of study, and (5) species in corn and soybean would be favored

TABLE 1 | Mean number of sampling occasions per year in alfalfa, winter wheat, spring grains and restored prairie (sweepnet samples) and minutes sampled in corn and soybeans (timed visual searches) from 2007 to 2020.

Habitat	Mean no. samples per year (range)	Mean no. per year
Alfalfa	8.4 (3–12)	7092.9 sweeps
Winter wheat	5.7 (4–8)	4585.7 sweeps
Spring grains	5.5 (3–8)	4628.6 sweeps
Restored prairie	8.5 (0–12)	3942.9 sweeps
Corn	6.1 (4–8)	329.9 min
Soybean	6.9 (4–9)	376.9 min

by the expanding land use for these two crops. To this end, we sampled five field crops and restored prairie over a 14-year period to assess coccinellid assemblages amid the changing landscape in eastern South Dakota. We also compared the sampling rate and composition of coccinellids in our study with earlier, long term studies from eastern South Dakota in order to assess our findings within a recent historical context.

METHODS

Study Area

Coccinellids were sampled from mid-May through early September during the years 2007 through 2020 within a roughly 8-km^2 area of Brookings Plat, Brookings County, in east-central South Dakota, USA. This area was within a triangle bounded by the three sampling sites in east-central South Dakota used by Kieckhefer et al. (1992) and Elliott et al. (1996) for previous long-term monitoring of coccinellid populations. Our study sampled six habitats, i.e., five types of field crops and restored prairie. Sample sites for field crops consisted of 0.5 to 2-ha plots that were located at the north end of the 8-km^2 area and roughly 1km N of the city of Brookings, South Dakota. The five field crops included a perennial crop, i.e., alfalfa, and four annual row crops: spring-seeded small grains (wheat in 2007, wheat and oats in 2008, and oats in following years), winter wheat (fall-planted), corn, and soybean. Alfalfa plots ranged from 2 to 6-years-old. The crops were grown using standard agronomic practices for the region (Pikul et al., 2008) that included fertilization of annual crops and one or more herbicide applications in the first half of each growing season. None of the plots received foliar insecticide spray, but corn seed was typically treated with a neonicotinoid insecticide, which had become routine practice since 2004 [USGS (U.S. Geological Survey), 2014]. Three replicate plots of each field crop were typically sampled, but in 2007–2009 only 1 or 2 plots of a particular crop were available (**Table 1**, **Supplementary Table 2**).

Two restored prairie tracts that consisted of native grasses and forbs were sampled over the course of the study. A list of dominant plants at the prairies are listed in **Supplementary Table 3**. The first, "Brookings Prairie," was a 16-ha tract that was sampled from 2007 through 2013. It was the nearest, suitable native prairie tract during that period and located at the south end of the 8-km^2 area. However, by 2014, the first prairie tract had accumulated excessive dry plant residue due to lack of burning and grazing. In 2014, sampling was switched to a 2-ha tract of restored prairie established in 2000 at the north end of the 8-km^2 area ("North Prairie") and that tract was spring burned every 2–3 years.

Sampling Procedures

Sampling of coccinellids was conducted by sweepnetting spring grains, winter wheat, alfalfa, and prairie and by timed searching of corn and soybean. Searches and sweeps were conducted between 09:00 and 16:00 h when sunny to mostly sunny and wind speed $<32\,\text{km h}^{-1}$ (Kieckhefer et al., 1992). Sampling was conducted in a manner that avoided casting a shadow on the sample area to avoid startling and dispersing coccinellids (Hesler and Kieckhefer, 2008). Sweepnetting consisted of 180°-sweeps with a 38-cm (diam.), mesh net along 50-m transects within individual plots of spring grain, winter wheat, alfalfa, and restored prairie. Three hundred sweeps were made per plot on each sampling date in the field crops, whereas sampling consisted of 300 (2007–2008) or 900 sweeps per date (other years) at Brookings Prairie and 300 sweeps at North Prairie. We made 900 sweeps in the Brookings Prairie (16 ha) in later years to equate to the 300 sweeps × 3 replicates in other habitats, but the smaller size of the North Prairie (2 ha) constrained us to 300 sweeps per date. Sweep samples from each plot were placed into a plastic bag and stored in a freezer until coccinellids could be identified and counted at a later date. Adults were identified to species based on descriptions in Gordon (1985) and Gordon and Vandenberg (1991). Immature coccinellids (larvae and pupae) were tallied without species identification.

Timed sampling consisted of a 20-min search per plot while walking between inner rows of corn or soybean plots and tallying all coccinellids by species seen on plants. Our sampling was biased toward searching for lady beetles in the mid- to upper canopy of corn and soybean, where a majority of aphids tends to be in these crops (Bing et al., 1999; McCornack et al., 2008; Prescott and Andow, 2016). As the season progresses, lady beetles can become increasingly difficult to see in the uppermost canopy of corn (over 2.5 m height) and the mid-canopy of soybean (density of foliage), thereby reducing sampling efficiency by an undetermined amount (Prescott and Andow, 2016). Identifications of adults were made in the field based on familiarity with species commonly encountered in our area.

Sampling among the different habitats varied during the season, similar to the timing of sampling in Kieckhefer et al. (1992) and Elliott et al. (1996). Alfalfa and winter wheat were the first habitats to be sampled each year, with first sample dates ranging from mid-May to early June. These were followed by availability of spring grains and prairie in early to mid-June. Alfalfa was often available for sampling throughout the summer, but its cutting and baling precluded sampling during some weeks. Dry conditions that prevented adequate regrowth of alfalfa occasionally delayed or prevented sampling it later in summer. Winter wheat and spring grains were sampled until their respective grain maturities in mid-summer. Corn and soybean were sampled in basically the latter half of summer, ranging from late June to early September, when prey of coccinellids were available in these crops.

Efforts were made to sample habitats weekly, and from one to four habitats may have been sampled in any given week. However, various abiotic conditions and logistical considerations imposed limitations on sampling frequency. For example, periods of excessive wind, extensive cloud cover, and rain, herbicide application, and competing workload occasionally precluded sampling within a week, resulting in different numbers of sample dates among habitats each year (**Table 1**, **Supplementary Table 2**).

Data Analyses

The numbers of adults with individual species and immature coccinellids in aggregate were summed across habitats, replicates, and years to determine their relative abundance. In addition, annual abundances by habitat were summed across species for adult and immature coccinellids and used to test whether annual abundance was at equilibrium within each habitat (Lamb et al., 2019). Trends in abundance over the 14-year sampling period were tested by using the slopes of a linear regression model relating annual abundance by time in years, with a significant positive or negative relationship between abundance and years indicating a lack of equilibrium in coccinellids within a particular habitat (Lamb et al., 2019). Tests of multiple species from a habitat were not considered independent, and therefore adjusted α values were used in individual tests (Hochberg, 1988).

Abundance was also averaged across all sampling dates for each habitat, and measurements were standardized by sampling method to account for unevenness in sampling frequency among habitats and years. The standardized measures of abundance were used to determine the breadth of habitat use for each species (Southwood and Henderson, 2000; Lamb et al., 2019). Breadth of habitat use was calculated by using the reciprocal Simpson-Yule index (Southwood and Henderson, 2000):

$$D = 1/\sum_{1}^{m} p_i^2,$$

where p_i is the proportion of species i in each of the $m \geq 2$ habitats. The value of D ranges from 1 when a species is present in only one of the habitats sampled and reaches a maximum of m when a species is distributed equally among the m habitats. Breadth in habitat use among coccinellids was calculated individually for adult species and for immatures across species and reported separately for the four habitats sampled by sweeping (alfalfa, winter wheat, spring small grains, and restored prairie) and the two sampled by timed searching (corn and soybean). As such, D could range from 1 to 4 for the group of habitats sampled by sweeping and from 1 to 2 for corn and soybean.

Average abundance of coccinellids over the 14-year period was also used to determine their overall rank in abundance by habitat. Rank abundance of species may be used as a measure to test for similarity, or concordance, in the structure of coccinellid assemblages among habitats (Southwood and Henderson, 2000; Legendre, 2005). Concordance was determined by calculating Kendall's coefficient (W, Kendall and Gibbons, 1990; Legendre, 2005; Zar, 2010) and accounted for zero abundance of a species and ties in rank abundance within a particular habitat (Legendre, 2005). The value of W may range from 0, when there is no correlation in ranks among habitats, to 1, when ranks completely agree. Calculation of Friedman's $\chi^2 = M(n - 1)W$ was used to determine if the concordance value differed significantly from zero (Zar, 2010). W is related to the pairwise Spearman correlations in rank abundance (Legendre, 2005). Hence, following a significant outcome for W, a Spearman correlation matrix was computed among the ranks for all habitat pairs using an adjusted α (Hochberg, 1988; Legendre, 2005), and the Spearman correlations were interpreted as similarity indices of rank abundance of coccinellids among habitats (Legendre, 2005). Spearman correlations were used as thresholds for developing a dendrogram to delineate clusters of habitats with concordant ranks of coccinellid assemblages (Southwood and Henderson, 2000; Legendre, 2005; Honek et al., 2014).

Finally, to evaluate abundance of coccinellids in our study against that reported from previous studies in the region, we compared the rates at which adult coccinellids were sampled between our study and that of Elliott et al. (1996). Rates at which coccinellids were sampled by Elliott et al. (1996) were derived by using the total number of adult coccinellids sampled in each crop (their Table 2) and dividing that by the total amount of sampling within each crop, which was calculated by multiplying mean number of samples per year by mean number of sweeps per year (alfalfa and spring grains) or minutes of searching per year (corn) from their Table 1.

RESULTS

In all, 17,338 predacious coccinellids comprising 10 species were sampled (**Table 2**). *Hippodamia convergens, Col. maculata lengi, Har. axyridis*, *Coc. septempunctata, Hip. parenthesis*, *Hip. tredecimpunctata tibialis*, and *Cyc. munda* collectively constituted nearly all individuals sampled, and each of these species occurred in all habitats. Two of the thee invasive species, *Har. axyridis* and *Coc. septempunctata*, ranked third and fourth in abundance, respectively. The non-native *Hip. variegata* accounted for only nine of all coccinellids sampled, and it was found in alfalfa, winter wheat, and soybean. Two *Hippodamia glacialis* were sampled from alfalfa, and a single *Anisosticta bitriangularis* was sampled from restored prairie.

Trends in Coccinellid Abundance

As a group, average abundance of coccinellids fluctuated considerably from year to year in each of the six habitats. All habitats were associated with declining trends in annual abundance of total adult coccinellids (i.e., across species) over the 14 years of sampling (**Table 3**), with significant negative regressions in annual abundance among years for adult and immature coccinellids in corn and adults in soybean (**Figure 1**). As a group, native adult coccinellids showed a significant declining trend in corn but not in other habitats, whereas trends for non-native adult coccinellids were non-significant in all habitats (**Table 3**). Individual species of either native or

TABLE 2 | Number of predacious coccinellids sampled from 2007 to 2020 in field crops and restored prairie in east-central South Dakota, USA.

Species	Abundance	(Percent)	Habitats[a]
Adults			
Hippodamia convergens	4,817	(44.4)	A, W, G, P, C, S
Coleomegilla maculata lengi	1,964	(18.1)	A, W, G, P, C, S
Harmonia axyridis	1,447	(13.3)	A, W, G, P, C, S
Coccinella septempunctata	1,061	(9.8)	A, W, G, P, C, S
Hippodamia parenthesis	976	(9.0)	A, W, G, P, C, S
Hippodamia tredecimpunctata tibialis	402	(3.7)	A, W, G, P, C, S
Cycloneda munda	162	(1.5)	A, W, G, P, C, S
Hippodamia variegata	9	(<0.1)	A, W, S
Hippodamia glacialis glacialis	2	(<0.1)	A
Anisosticta bitriangularis	1	(<0.1)	R
All adults	10,841	(100.0)	A, W, G, P, C, S
Immatures[b]	6,497		A, W, G, P, C, S
Total	17,338		

[a] *A, alfalfa; W, winter wheat; G, spring grains; P, restored prairie; C, corn, S, soybean.*

[b] *Larvae and pupae.*

non-native adult coccinellids showed no significant trends in abundance over the years (**Table 3**).

Abundance and Evenness of Coccinellids Among Habitats

In habitats sampled by sweepnetting, abundances of adult coccinellids by individual species were generally highest in alfalfa and winter wheat, the two earliest crops, and followed in abundance by spring grains and then restored prairie, except that *Cyc. munda* was more abundant in prairie than spring grains (**Table 4**). This trend was reflected by evenness values that ranged from moderately high for *Cyc. munda* ($D = 2.91$) to moderately low for *Har. axyridis* ($D = 2.09$). Similarly, immature coccinellids had moderately low evenness among habitats ($D = 2.33$), reflected by relatively high abundance in alfalfa and winter wheat, moderate abundance in spring grains, and low abundance in restored prairie.

Some adult coccinellids were particularly uneven ($D = 1.03$–1.48) between habitats sampled by timed searches, with abundance of most species skewed toward soybean, except that *Col. maculata lengi* had higher abundance in corn and *Cyc. munda* ($D = 1.86$) was sampled roughly equally in both crops (**Table 4**). Immature coccinellids reflected the general trend of adults, with particularly high abundance in soybean and relatively low abundance in corn ($D = 1.25$).

Concordance in Species Abundance Among Habitats

The rank abundances of coccinellid species were significantly though only moderately concordant among habitats ($W = 0.541$; $\chi^2 = 19.5$, $df = 6$, $p = 0.003$). *Post-hoc* Spearman correlation tests (**Supplementary Table 4**) revealed that concordance was derived mainly from significantly high similarity in rank abundances of adult coccinellids between alfalfa and winter wheat (**Figure 2**). Spring grains had a high but non-significant similarity to the alfalfa-winter wheat group. Likewise, restored prairie had relatively high but non-significant similarity in species composition to the alfalfa-small grains group. Corn and soybean showed a modest, non-significant similarity in rank abundance that was distinct from that of alfalfa, winter wheat, spring grains, and restored prairie.

Comparison of Sampling Rates With Earlier Reports

A comparison of the rates of at which coccinellids were sampled between our study and that of Elliott et al. (1996) showed that we sampled adult coccinellids at lower rates that varied by crop (**Table 5**). Specifically, we sampled coccinellids at 73.7% of their rate in alfalfa (adult coccinellids per 100 sweeps), roughly one-quarter of their rate in small grains (adults per 100 sweeps), and only 5.6% of that in corn (per 10 min. search).

DISCUSSION

Native and Non-native Species Composition

The six habitats in our study shared seven coccinellid species that accounted for >99% of all coccinellids sampled (**Table 2**). Kieckhefer et al. (1992) also found that seven coccinellid species were predominant in alfalfa, spring grains, and corn in east-central South Dakota. Five species were common to their study and ours (*Col. maculata lengi*, *Cyc. munda, Hip. convergens, Hip. parenthesis*, and *Hip. tredecimpunctata tibialis*), whereas *A. bipunctata* and *Coc. transversoguttata richardsoni* were only found in their studies. Elliott et al. (1996) subsequently reported establishment of *Coc. septempunctata* in alfalfa, spring grains and corn in east-central South Dakota, and detected significant decreases in abundance of *A. bipunctata* and *Coc. transversoguttata richardsoni* following its establishment. There have been no subsequent reports of *A. bipunctata* from eastern

TABLE 3 | Slopes of linear regressions of annual abundance of adult coccinellids by species groups across years of sampling in six habitats.

Species group	Statistic	Alfalfa	Winter wheat	Spring grains	Restored prairie	Corn	Soybean
All adults	Slope	−0.059	−0.271	−0.130	−0.177	−0.097	−1.116
	p	0.76	0.43	0.19	0.12	0.013	0.04
Native	Slope	0.002	−0.284	−0.081	−0.136	−0.083	−0.256
	p	0.99	0.39	0.33	0.17	0.023	0.21
	Hochberg α[a]	0.05	0.025	0.05	0.05	0.025	0.05
Non-native	Slope	−0.061	0.014	−0.049	0.013	−0.014	−0.859
	p	0.06	0.56	0.10	0.15	0.11	0.051
	Hochberg α	0.025	0.05	0.025	0.025	0.05	0.025
Coccinella septempunctata	Slope	−0.056	0.015	−0.044	0.090	–[b]	−0.021
	p	0.047	0.50	0.13	0.07	–	0.67
	Hochberg α	0.007	0.017	0.017	0.01	–	0.05
Coleomegilla maculata lengi	Slope	−0.042	−0.142	0.014	−0.141	−0.079	−0.005
	p	0.40	0.36	0.66	0.09	0.031	0.40
	Hochberg α	0.013	0.013	0.05	0.013	0.013	0.025
Cycloneda munda	Slope	−0.003	0.007	–	0.006	−0.002	−0.025
	p	0.67	0.29	–	0.12	0.49	0.039
	Hochberg α	0.025	0.01	–	0.017	0.025	0.008
Harmonia axyridis	Slope	−0.005	–	–	–	−0.010	−0.840
	p	0.47	–	–	–	0.19	0.055
	Hochberg α	0.017	–	–	–	0.017	0.01
Hippodamia convergens	Slope	0.048	0.0002	−0.040	0.001	−0.001	−0.220
	p	0.70	0.99	0.52	0.98	0.86	0.27
	Hochberg α	0.05	0.05	0.025	0.05	0.05	0.017
Hippodamia parenthesis	Slope	0.034	−0.011	−0.027	−0.007	–	–
	p	0.35	0.69	0.031	0.48	–	–
	Hochberg α	0.010	0.025	0.01	0.025	–	–
Hippodamia tredecimpunctata tibialis	Slope	−0.035	−0.125	−0.025	–	–	−0.005
	p	0.08	0.054	0.13	–	–	0.11
	Hochberg α	0.008	0.008	0.017	–	–	0.013
Immatures[c]	Slope	−0.080	−0.244	−0.024	−0.021	−0.155	−1.069
	p	0.71	0.54	0.11	0.09	0.005	0.10

[a] *Hochberg (1988) correction applied for multiple testing of individual species within habitats.*

[b] *Dash indicates too few beetles for analysis.*

[c] *Larvae and pupae across species.*

South Dakota, and *Coc. transversoguttata richardsoni* has not been reported there since 1993 (Hesler et al., 2005; Hesler and Kieckhefer, 2008; Hesler and Petersen, 2008). Thus, it is likely that *A. bipunctata* and *Coc. transversoguttata richardsoni* have become locally extinct in east-central South Dakota.

Three non-native species (*Coc. septempunctata*, *Har. axyridis*, and *Hip. variegata*) comprised 23.1% of all coccinellids that we sampled. *Coc. septempunctata*, the first invasive to establish in eastern South Dakota, remained relatively abundant during the 14 years of our study, consistent with our prediction. *Harmonia axyridis*, first detected in east-central South Dakota in 1996, had become third-most abundant coccinellid overall, which is similar to that reported from earlier surveys following its establishment in east-central South Dakota (Hesler and Petersen, 2008; Hesler, 2014). Thus, abundance of *Har. axyridis* was also in accord with our predictions, but its abundance pattern depended on habitat (see below). In contrast, only seven *Hip. variegata*, the latest non-native to arrive in South Dakota (Hesler and Lundgren, 2011), were sampled in our study, contrary to our prediction that it would establish at least low abundance. Many non-native species, such as *Hip. variegata* currently in eastern South Dakota, persist indefinitely at very low population levels and may be classified as "sleeper populations" due to undetermined biotic or abiotic conditions that limit their populations (Spear et al., 2021).

Hippodamia variegata has been associated with a negative impact on population levels of a native species, *Cyc. munda* (Lamb et al., 2019). *Cycloneda munda* accounted for 1.5% of coccinellids sampled in our study, comparable to its previous 1% abundance (Kieckhefer et al., 1992). Furthermore, it showed no significant population trends in any of the six habitats that we surveyed (**Table 4**). Nonetheless, additional research is needed to monitor the abundance of *Hip. variegata* and for

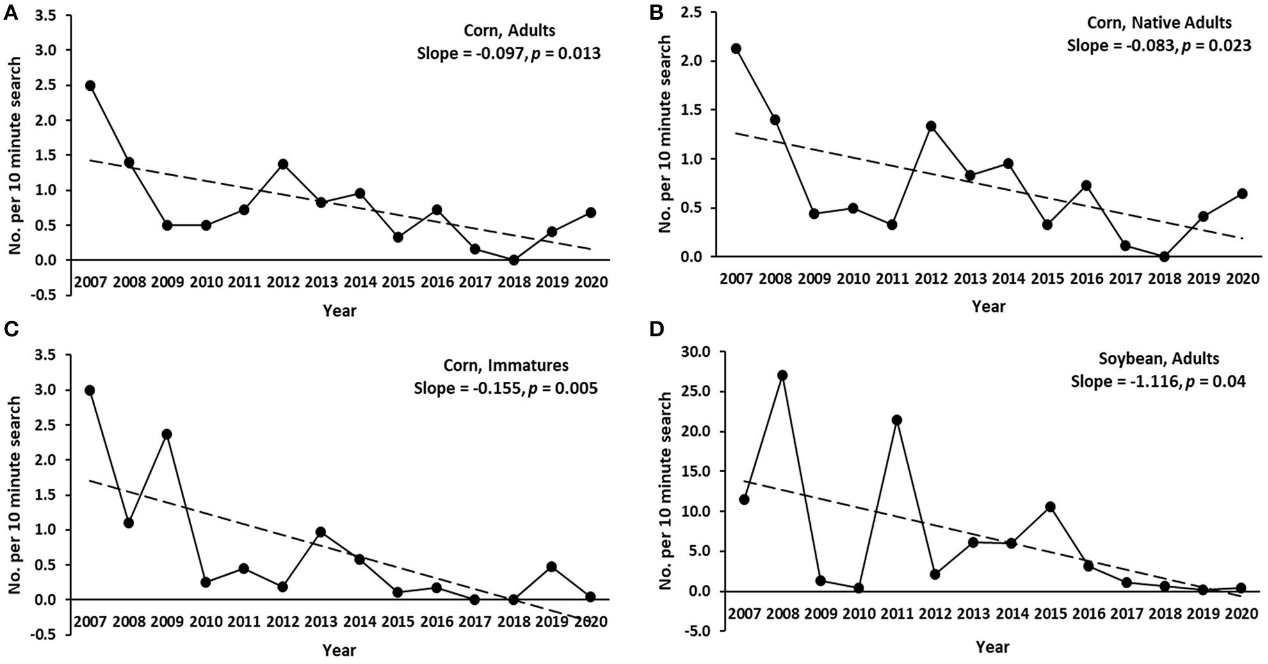

FIGURE 1 | Significant declining trends in annual coccinellid abundance (average no. of coccinellids per 10-min search) in corn and soybean in east-central South Dakota, with slopes and p-values of linear regressions for the respective trend lines. **(A)** Corn, adults across species. **(B)** Adults of native species. **(C)** Corn, immatures (larvae and pupae) across species. **(D)** Soybean, adults across species.

TABLE 4 | Abundance of predacious coccinellids and evenness (*D*) in their habitat use among selected field crops and restored prairie in east-central South Dakota, 2007–2020.

	Average no. per 100 sweeps[a]					Average no. per 10 min of searching[a]		
Species	**Alfalfa**	**Winter wheat**	**Spring grains**	**Restored prairie**	***D***	**Corn**	**Soybean**	***D***
Adults								
Coccinella septempunctata	0.534	0.312	0.130	0.050	2.62	0.006	0.415	1.03
Coleomegilla maculata lengi	0.650	1.131	0.449	0.067	2.77	0.491	0.074	1.29
Cycloneda munda	0.063	0.078	0.006	0.034	2.91	0.019	0.034	1.86
Harmonia axyridis	0.063	0.017	0.011	0.005	2.09	0.048	2.542	1.04
Hippodamia convergens	1.625	2.076	0.764	0.152	2.82	0.065	2.394	1.05
Hip. parenthesis	0.629	0.382	0.074	0.089	2.48	0.004	0.013	1.48
Hip. tredecimpunctata tibialis	0.122	0.316	0.088	0.005	2.30	0.006	0.028	1.43
Immatures[b]	1.945	3.042	0.522	0.056	2.33	0.483	3.784	1.25

[a] *Across years.*

[b] *Larvae and pupae across all species.*

potential adverse impacts it may have on *Cyc. munda* and other coccinellids in eastern South Dakota.

Abundance Among Habitats

Although all six habitats shared the seven most abundant coccinellids, different patterns of their abundance were evident among habitats. *Cycloneda munda* was consistently sampled at low but even rates among habitats, and four species (*Col. maculata lengi*, *Coc. septempunctata*, *Hip. convergens*, and *Hip. parenthesis*) were sampled at high to moderately high rates in four of the six habitats, consistent with their generalist use patterns in previous studies (Elliott et al., 1996; Turnock et al., 2003; Hesler and Kieckhefer, 2008). In comparison, *Har. axyridis* was sampled at high rates mainly in soybean, indicative of a high degree of specialization among the six habitats in this study. This high degree of habitat specialization by *Har. axyridis* contrasts with other reports of its relatively even (Lamb et al., 2019) and general habitat use (Koch, 2003; Hesler et al., 2004; Hesler and Kieckhefer, 2008).

Alfalfa, winter wheat, and spring grains were similar in rank abundance, but coccinellids were sampled at higher rates in alfalfa and winter wheat. There was a lack of similarity in rank

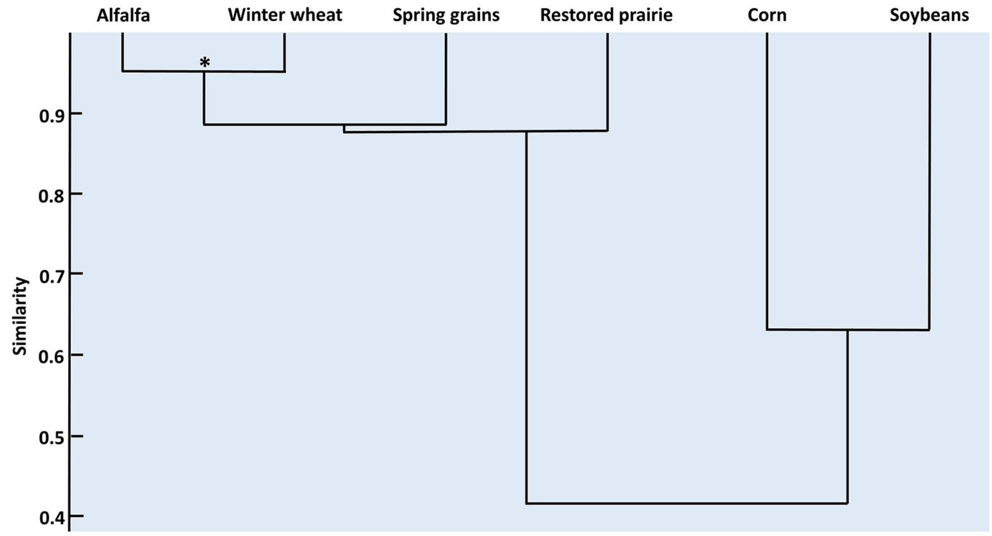

FIGURE 2 | Dendrogram depicting the relationships among coccinellid assemblages in six habitats in east-central South Dakota based on correlations of species rank abundance. $^*p < 0.001$.

TABLE 5 | Sample rates of adult coccinellids among habitats in two studies from east-central South Dakota, USA.

Habitat	Current study[a]	Elliott et al., 1996[b]	Ratio between studies
Number per 100 sweeps			
Alfalfa	3.69	5.01	0.737
Spring grains	1.52	5.89	0.258
Winter wheat	4.32	–[c]	–
Restored prairie	0.39	–[c]	–
Number per 10 min			
Corn	0.64	11.4	0.056
Soybean	5.50	–[c]	–

[a] *Sample period: 2007–2020.*

[b] *Sample periods: 1974–1985, 1988–1992.*

[c] *Habitat not sampled.*

abundance of coccinellids between alfalfa and winter wheat and the other four habitats. Coccinellids were abundant in soybean, but particularly low in abundance in corn and restored prairie. Previous long-term studies of coccinellids in east-central South Dakota found correlations in rank abundance of adult and immature coccinellids were strongest between alfalfa and spring grains, intermediate between spring grains and corn, and weakest between alfalfa and corn (Kieckhefer et al., 1992). However, short-term studies in such habitats within the region have failed to find similarities in coccinellid abundance among crops (Hesler and Kieckhefer, 2008; Prescott and Andow, 2016), which may be indicative of the large year-to-year fluctuations in abundance of coccinellid species and temporal sensitivity in the analysis of coccinellid assemblages (Elliott and Kieckhefer, 1990b; Elliott et al., 1996).

Our results showed declines in coccinellid abundance during the tenure of our study and also decreased abundance when compared to a previous long-term survey in east-central South Dakota (Elliott et al., 1996). Declines in coccinellid abundance during our study were significant for both adult and immature coccinellids in corn and for adults in soybean. Decreased abundance relative to earlier studies was most striking in corn, substantial in spring grains, and notable, though less severe, in alfalfa. Decreased prey availability in various crops might have been responsible for declining trends in coccinellid abundance during our study and for discrepancies in sample rates between our study and Elliott et al. (1996). However, it should be noted that neither study quantified prey, but nonetheless, discussion about the prey within our crop plots and information from recent studies in the various crops may help to explain trends in coccinellid abundance between studies.

Two common pests of alfalfa between our study and Elliott et al. (1996) have been the pea aphid (*Acyrthosiphum pisum*) and the alfalfa weevil (*Hypera postica*), which occasionally reach economic levels in eastern South Dakota (Catangui et al., 2002). Both are prey for coccinellids, but aphids are preferred over weevil larvae (Kalaskar and Evans, 2001). We did not find published data regarding recent long-term trends in alfalfa weevil populations in South Dakota, but pea aphid abundance declined from 1 to 30% between 2005 and 2019 as measured by a suction trap network across the Midwestern U.S. that included eastern South Dakota (Crossley et al., 2021). Day and Tatman (2006) suggested that widespread establishment of *Aphidius* spp. of parasitoid wasps in the U.S. may have reduced pea aphid populations in alfalfa, with subsequent reductions in coccinellids, although they acknowledged this has not been documented by published studies.

In spring grains, various species of cereal aphids (Hemiptera: Aphididae) have been the main pests, primarily as vectors of viruses that are responsible for diseases generically known as barley yellow dwarf (Hesler et al., 2018). However, these aphids rarely build sufficient numbers to cause yield loss directly in east-central South Dakota (Hesler et al., 2000, 2018). Abundance, as measured by suction traps, showed a decline of 1–10% for two common cereal aphids (*Rhopalosiphim padi* and *Schizaphis graminum*) but increases ranging up to 29% for a third species (*Sitobion avenae*) (Crossley et al., 2021).

Early-season colonization of alfalfa, spring grains and winter wheat by coccinellids may be a trade-off between crop phenology and crop extensiveness. In terms of phenology, winter wheat is available for colonization by coccinellids and their prey earlier than spring grains. However, spring grains have historically been roughly six times more extensive than winter wheat in the agricultural landscape of east-central South Dakota, although both types of crops have steadily declined as a proportion of farmland over the last four decades [NASS (National Agricultural Statistics Service), 2021]. Thus, the much greater amount of spring grains may have considerably offset the earlier availability of winter wheat, such that in the study by Elliott et al. (1996), fields of spring grains would have been a major early-season sink for coccinellids and their prey. In contrast, spring grains and winter wheat made up small but comparable land uses at our research site, and thus favored coccinellid colonization of winter wheat due to its earlier availability. Indeed, the combined rate of adult coccinellid capture in spring grains and winter wheat (5.8%) in our study was comparable to that by Elliott et al. (1996) in spring grains alone (5.9%) (**Table 5**).

The corn leaf aphid (CLA, *Rhopalosiphum maidis*) and European corn borer (ECB, *Ostrinia nubilalis*) have historically been common pests of corn in the Midwestern U.S., including South Dakota (Bing et al., 1999; Hutchinson et al., 2010). We infrequently observed CLA and the ECB or its damage in eastern South Dakota corn fields during the period of our study. Corn hybrids with ECB resistance have become increasingly common since 1996 in the Midwestern U.S., including eastern South Dakota (Fernandez-Cornejo et al., 2014, Perry and Moschini, 2020), and the widespread planting of ECB-resistant corn has decreased levels of this pest across the Midwest due to areawide suppression (Hutchinson et al., 2010).

The near absence of CLA in our corn plots was first noticed in 2010, which corresponded temporally to lower annual abundance of coccinellids since 2009 in corn (**Figure 1**). Concurrently, CLA captures in suction traps declined 11 to 30% in the Midwest between 2005 and 2019 (Crossley et al., 2021). It was not clear why CLA populations decreased, but factors either within or outside of our study area may have been responsible. For instance, the development of corn hybrids highly resistant to CLA could have suppressed populations of this pest, but unlike ECB resistance, we have not found evidence that CLA resistance had been intentionally bred into commercial corn hybrids used in the USA.

Alternatively, factors outside of our study area may have led to decreased CLA infestations of corn in our study. CLA infestations in eastern South Dakota originate from immigrants of populations on crops such as sorghum in the southern United States (Irwin and Thresh, 1988). A recent upsurge in insecticide applications against the invasive sugarcane aphid (*Melanaphis sacchari*) on sorghum in Mexico and south-central U.S.A. (Bowling et al., 2016) may have concomitantly suppressed co-infestations by CLA and thus diminished migrant populations. Additional research is needed to delineate factors underlying the regional scarcity of CLA.

Soybean aphid had been a major pest of soybean in the Midwestern United States since 2000 (Ragsdale et al., 2011) and a major prey item of coccinellids in that crop (Fox et al., 2004; Schmidt et al., 2008). However, populations of soybean aphid have decreased substantially over the last 10–15 years in the region (Bahlai et al., 2015; Crossley et al., 2021; Hesler and Beckendorf, 2021), and this temporally corresponds to significant declines of adult coccinellids in soybeans in our study (**Figure 1**). Comparisons with earlier surveys of coccinellids in eastern South Dakota are not available because historically the crop lacked significant arthropod pests and robust predator populations (Ragsdale et al., 2011; Hesler, 2014).

Beyond considerations of prey availability in various crops, markedly lower sampling rates could have been due to factors such as field size and various landscape characteristics. Whereas our crop plots were generally between 0.5 to 2-ha, Elliott et al. (1996) sampled coccinellids from commercial crop fields, which were typically >10 ha in east-central South Dakota [NASS (National Agricultural Statistics Service), 2021]. Literature on the effect of field size on coccinellid abundance is sparse and often confounded with larger landscape factors. Some studies suggest that densities of coccinellids are lower near edges compared to the interior of fields (Olson and Andow, 2008; Caballero-López et al., 2012). Thus, while we avoided sampling from edges of our plots, their greater edge-to-area ratio could have lowered coccinellid densities amid the interiors. Among many tradeoffs that may be considered in designing experiments, plot size may need to be accounted for in research on coccinellid assemblages.

Landscape factors may have influenced coccinellid abundance in our study. For instance, over the last 40 years, winter wheat and spring grains have been steadily replaced by corn and soybeans, and these latter two crops now comprise a large majority of the cropland in eastern South Dakota [Dumke and Dobbs, 1999; Johnston, 2013; Wright and Wimberly, 2013; NASS (National Agricultural Statistics Service), 2021]. The intensified planting of corn and soybean decreases the overall heterogeneity of the landscape, and studies have generally shown that diminished landscape heterogeneity is associated with decreased abundance of natural enemies, and particularly (native) coccinellids (e.g., Elliott et al., 2002; Landis et al., 2008; Grez et al., 2014, 2021; Woltz and Landis, 2014).

It is unclear whether adequate alternative habitat can be exploited by coccinellids to compensate for the impact of increased corn and soybean plantings with lower pest levels. All of the coccinellids found in our study are considered generalists (Elliott et al., 1996; Hesler and Kieckhefer, 2008), and thus may adapt to alternative habitats, although the spillover of agrobiont species into non-agricultural habitats can impact native non-pest herbivores (Koch, 2003; Rand and Louda, 2006). Some have suggested that alternative habitat such as restored tallgrass prairie may serve as refuges for coccinellids (Hesler and Petersen, 2008; Diepenbrock and Finke, 2013), but we found relatively low numbers of coccinellids in prairie in our study and no evidence that they shifted to prairie as their populations declined in corn and soybean. Thus, the types of alternative, late-season habitat that may be colonized by coccinellids remains undetermined for east-central South Dakota.

Although the six habitats sampled in our study largely shared coccinellid species, stark distinctions in rank abundance of coccinellids among corn and soybean and a group consisting of alfalfa, winter wheat, spring grains and prairie suggest that species may be, or already have been, impacted differentially by the expansion of corn and soybean plantings in east-central South Dakota. The impacts may differ among species. For instance, *Har. axyridis* was a specialist in soybean and present at relatively low numbers in early-season habitats and corn. Thus, it likely has alternative, perhaps arboreal, early-season habitat, but it will have to find additional late-season habitat. Decreased abundance of *Har. axyridis* may have benefits in terms of limiting its intra-guild predation within the coccinellid assemblage (Gardiner et al., 2011; Koch and Costamagna, 2017) and by reducing the nuisance of its mass overwintering in homes (Koch, 2003).

Native species made up the majority of coccinellids in corn and experienced significant declines in this crop, whereas trends for non-native species were non-significant in corn. A species such as *Col. maculata lengi* may be expected to persist in corn irrespective of prey levels because of its ability to complete development solely on corn pollen (Smith, 1960). Nevertheless, its population trend was also negative and marginally significant ($p = 0.03$, adjusted $\alpha = 0.013$), and this raises questions about what factors contributed to declines that have affected native coccinellids and whether they are prey-dependent or not.

The significant declines of native species in corn may be especially impactful on *Hip. tredecimpunctata tibialis*. It was historically one of the three most abundant coccinellids in alfalfa, spring grains, and corn (Olsen, 1971; Kieckhefer et al., 1992; Elliott et al., 1996) but one of the least abundant species across habitats in our study. Numbers of *Hip. tredecimpunctata tibialis* were particularly low in late season crops, with only three adults sampled from corn (all in 2008) and 15 from soybean in six of the 14 years, suggesting that its decline may have pre-dated our study. We suggest that *Hip. tredecimpunctata tibialis* is a specific candidate for further monitoring, particularly in late-season alternative habitats, given its very low numbers in corn and soybean in our study.

IMPLICATIONS FOR COCCINELLID POPULATIONS

Long-term declines of native coccinellid populations have now been documented in many regions (Harmon et al., 2007). Often, the declines are associated with the establishment of invasive coccinellids species (Elliott et al., 1996; Turnock et al., 2003; Alyohkin and Sewell, 2004; Honek et al., 2016; Brown and Roy, 2018), though not in all cases (Harmon et al., 2007; Evans et al., 2011). In our study, declines were generally observed for coccinellid assemblages over 14 years in corn and soybean, with significant declines for native species as a group in corn. In addition, sampling rates were lower compared to an earlier study (Elliott et al., 1996). However, direct effects of non-native coccinellids on the declines were not apparent. Although it is unclear what factors may actually underlie the lower coccinellid population trends observed in our study, the trend of increased amounts of corn and soybean could have compounding effects that result in major impacts on coccinellid populations in east-central South Dakota and perhaps elsewhere. First, planting corn and soybean at the expense of crops such as alfalfa and small grains directly diminishes availability of early-season habitat for coccinellids, where they have historically built up populations while providing biocontrol services. Second, while diminished pest levels in corn and soybean undoubtedly benefit production of these crops, vast areas of cropland devoid of suitable prey will decrease late summer populations of coccinellids and subsequently reduce overwintering populations that would emerge the following spring to colonize (early-season) crops. Thus, the annual cycle of coccinellids in South Dakota cropland could be severely disrupted both early and late in the season.

Various measures may be implemented to counter the decrease in landscape diversity due to widespread planting of corn and soybean in east-central South Dakota. Such measures include tailoring economic incentives for farmers to plant a wider diversity of crops and promote preservation of natural and semi-natural areas that may serve as refuges for coccinellids and other beneficial insects (Maisashvili et al., 2020). Measures that increase within-field diversity include leaving patches of plants that provide alternative, non-pest prey and the planting of cover crops that promote coccinellids and other beneficial insects but do not support crop pests (Schellhorn et al., 2015; Lundin et al., 2019). However, much of the cover crop research in northern Great Plains cropping systems has focused on soil health parameters, and additional research is needed to determine cover crops that may specifically provide resource continuity for coccinellids in the latter half of the growing season (Schellhorn et al., 2015). In summary, additional long-term research is needed to determine the factors underlying declines in eastern South Dakota, the extent to which various coccinellid species will adapt to changing conditions within crop fields and the general landscape of east-central South Dakota, and ways to sustain coccinellid populations in and around crop fields.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

LH conceived of the study, analyzed the data, and wrote the manuscript. LH and EB co-led data collection. EB managed the data and contributed to making figures. All authors contributed to the drafts and gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcosc.2021.742036/full#supplementary-material

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Warm Mesoclimate Advances the Seasonal Dynamics of *Harmonia axyridis* in Urban Habitats

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In 2016–2019, seasonal changes in the abundance of the harlequin ladybird *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) were established in the city center of Prague, Central Europe, and in its outskirts. Adults were sampled from lime trees (*Tilia* spp.) at regular intervals throughout the growing season. The abundance of *H. axyridis* paralleled the course of abundance of its prey, the aphid *Eucallipterus tiliae* L., which peaks either early or late in the season. As a result, the seasonal dynamics of *H. axyridis* were unimodal, with a peak in the early (late June—early July of 2017 and 2019) or late (late July—mid-September of 2016 and 2018) period of the season. In the early period, there was a small (1–4 days) difference in the timing of the peak of *H. axyridis* between the city center and the outskirts. In the late period, the peak occurred significantly earlier (by 13–21 days) in the city center due to the warmer climate there than in the outskirts. The difference in the timing of the population peak between both locations disappeared after recalculating the calendar to thermal time (number of day degrees above 10.6°C thresholds elapsed from the end of *H. axyridis* hibernation). The warm mesoclimate of the city center advances the seasonal dynamics of *H. axyridis*, contributing to the success of this invasive species in urban habitats.

Keywords: urban habitats, invasive, ladybirds, prey, temperature, mesoclimate, seasonal dynamics

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INTRODUCTION

In Central Europe, *Harmonia axyridis* (Pallas) (Coleoptera, Coccinellidae) is a dominant invasive ladybird, although it is only a recently established component of aphidophagous coccinellid communities (Roy et al., 2016). This species occurs in urban (Adriaens et al., 2008; Purse et al., 2015; Mukwevho et al., 2017), agricultural (Musser and Shelton, 2003; Lucas et al., 2007; Riddick, 2017), and forest areas (Vandereycken et al., 2012) on trees, herbs and crops populated by a variety of aphid species. This adaptability to diverse habitats is due to the plasticity of its seasonal cycle. Overwintering adults leave the hibernation sites in April. During the vegetative season, 2–3 incomplete generations of offspring may be produced, depending on temperature. In the autumn, adults of all generations enter dormancy and overwinter together (Honek et al., 2019b). Dispersion of adults into and using host stands for reproduction is opportunistic since adults always occupy stands with high prey abundance (Honek et al., 2019b). Due to its plastic life cycle and high adaptability to local conditions, *H. axyridis* is a suitable subject for studying the effects of urban and rural environments on the life cycle (Dudek et al., 2015; Sloggett, 2017; Honek et al., 2018b).

Among the characteristics that differentiate urban and rural areas is a difference in their mesoclimate, which is typically warmer in city centers than in the outskirts (Bonan, 2002). As temperature determines the rate of development of exotherms, we hypothesize that differences in mesoclimate affect the pace of life of *H. axyridis* and predict that (i) the peak abundance will occur earlier in the city center than in the outskirts and (ii) this difference will level off if population development is plotted against thermal time. This hypothesis was tested in 2016–2019, when populations of *H. axyridis* were sampled from *Tilia* sp. infested with aphids in the city center and on the outskirts of Prague.

MATERIALS AND METHODS

Sampling

The abundance of *H. axyridis* was recorded in 2 areas situated at ∼10 km distance in the western part of Prague, Central Europe. The beetles were sampled at 8 sites on the outskirts (situated between 50.081–50.094 N and 14.263–14.336 E) and at 4 sites in the city center (50.072–50.075 N, 14.454–14.465 E). The tree stands at particular sites consisted of $\geq$ 5 lime trees (*Tilia cordata* Mill. or *Tilia platyphyllos* Scop.) infested with the aphid *Eucallipterus tiliae* (L.) (Sternorrhyncha: Aphididae). This aphid species has a single peak of abundance, the timing of which varies from year to year (Dixon and Barlow, 1979; Dahlsten et al., 1999).

The lime trees were sampled throughout the whole period when *H. axyridis* adults were present on the lime trees (May to October) at 10-day intervals. Beetles were swept from the canopy up to a height of approximately 3 m. Sampling was standardized using a standard sweep net (35 cm diameter, 140 cm handle) operated by J.S. (city center) and A.H. (outskirts). All the days on which samples were collected were sunny and calm. The sampling (80–200 sweeps at each site) was performed, and *H axyridis* abundance (number of adults caught per 100 sweeps) was established between 08.00 and 18.00 h. The abundance of aphids was classified according to an earlier elaborated scale (Honek et al., 2019b) into 4 degrees as follows based on the aphid counts: (1) no aphids, (2) $<$ 0.002 aphids per leaf (aphids in swept material but no individuals found on 500 leaves), (3) $\geq$ 0.002 to 1.0 aphids per leaf, and (4) $>$ 1.0 aphids per leaf. The difference in urbanization at the outskirts and city center areas was quantified as the percentage of the surrounding area (a circle with a 500-m radius centered at each sampling site) covered by impervious surfaces (buildings, streets, parking places, etc.) (Honek et al., 2018b). These values were 39 ± 3.0% in the outskirts and 71 ± 5.6% in the city center and were determined using maps available at https://en.mapy.cz.

Air temperature at 2 m above the ground was used to calculate the thermal time. The data for the outskirts were taken from the meteorological station of the Crop Research Institute (50.085N, 14.298E), and the data for the city center were taken from the meteorological station Klementinum (50.086N, 14.416E). The thermal time (expressed in day degrees dd) for *H. axyridis* was calculated as the sum of effective temperatures (°C) above the 10.6°C lower development threshold (Honek et al., 2018a) starting on 20 April, the date *H. axyridis* left the hibernation sites (Honek et al., 2018a). The thermal time (expressed in day degrees dd) for *E. tiliae* was calculated as the sum of effective temperatures (°C) above the 5.0°C lower development threshold starting from the beginning of the year (Dixon et al., 1997; Brabec et al., 2014).

Data Analysis

For each year and site, the Julian day was determined when the maximum abundance of *H. axyridis* (population peak) was observed. Each year, the average terms of maximum abundance were calculated for the city center and the outskirts as the arithmetic mean (± SE) of the terms of population peaks at particular sites. Differences were tested using *t*-tests or (if the normality test failed) Mann-Whitney tests. Calculations were performed using SigmaStat 3.5 (Systat Software Inc, 2006).

For each site and year the relative frequency of *H. axyridis* was calculated so that the maximum abundance was rated 1 and the abundance on other dates as a proportion of this maximum. This data plotted against time (Julian day) was fitted by the Asymmetric Double Sigmoidal (ADS) function. Calculations were performed using TableCurve 2D (Systat Software Inc, 2002).

RESULTS

Each year, the course of change in the abundance of *H. axyridis* adults was unimodal. The term of peak abundance varied among years. In both areas, abundance peaked early in 2017 (22 June and 23 June) and 2019 (5 July and 9 July) and late in 2016 (29 August and 19 September) and 2018 (15 July and 28 July), with the peak occurring earlier every year in the city center than in the outskirts (**Figure 1**). The interval dividing the population peak in the city center and the population peak in the outskirts increased with the time that had elapsed since the beginning of the season (**Figure 2**). The difference was statistically significant in the years when abundance peaked late (2016 and 2018) (**Table 1**). The obvious difference on Julian days leveled off after the calendar time was transformed to thermal time (except 2017, when the population peak occurred very early). Differences in the timing of the population peak thus arose due to differences in the thermal conditions of the city center and the outskirts.

DISCUSSION

Mesoclimate Factors That Affect Population Development

Divergence in the quality of urban (downtown area) and rural (outskirt area) environments is well established and is associated with a variety of environmental characteristics. Urban centers differ from the outskirts in terms of excessive light pollution, decreased humidity, modified schedules of precipitation and variations in wind direction and speed (Bonan, 2002). The differences increase with the size of the town and are appreciable in cities whose human population exceeds 1 million (Karl et al., 1988), as is the case for Prague. Urban conditions affect

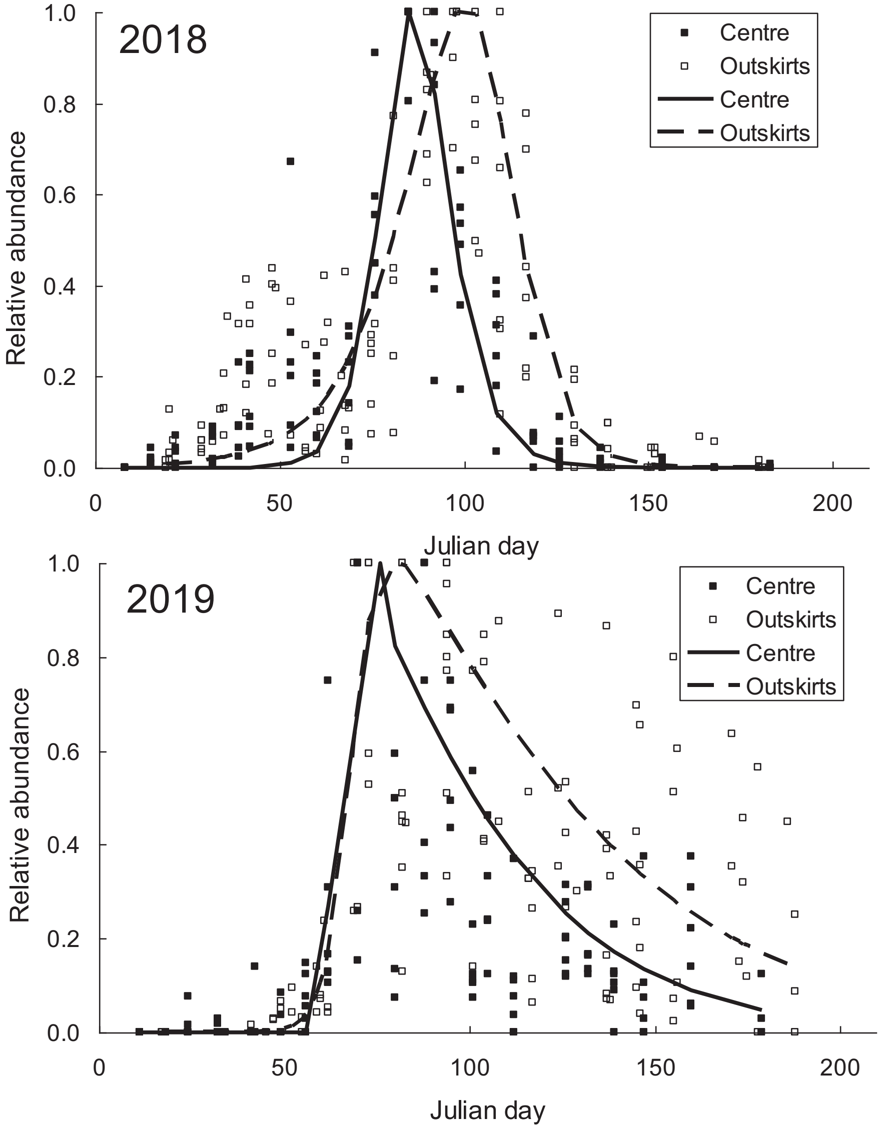

FIGURE 1 | Changes in the relative abundance of *Harmonia axyridis* in the city center and the outskirts of Prague plotted against calendar time (Julian days from leaving their hibernacula on 20 April). The maximum abundance at each locality is scored 1. The data were fitted to the asymmetric double sigmoidal (ADS) equation. The figure shows example of a year when population peak occurred late in the season (2018) and of a year when population peak occurred early in the season (2019).

vegetation (Pyšek, 1998), its insect phytophagans and their predators (Bolger et al., 2000; McIntyre, 2000; Pickett et al., 2001), as well as their pathogens (Dudek et al., 2017a,b). Apart from light pollution, divergent ambient temperature is the most important difference between urban and rural environments. The warm temperature of urban areas compared to that of rural areas accelerates the rate of life processes of exotherm organisms. Differences in their duration are compensated when calendar time is converted to thermal time (Trudgill et al., 2005).

The agreement between the term of the population peak in the city center and the term of the population peak in the outskirts resulting from this transformation (**Table 1**) indicates that the timing of peaks depends on the sum of a definite amount of heat. Increased ambient temperature accelerates the life processes of *H. axyridis* and shifts the peak of its seasonal cycle of abundance if sufficient prey is available to support an acceleration. This is a necessary consequence of the influence of urban centers on populations of exothermic organisms.

The effect of ambient temperature could be modified by thermoregulation, which can shift body temperature against ambient temperature, albeit slightly. A decrease in body temperature can be achieved by searching for and hiding in cool places. Coccinellids living in the city center on trees surrounded by buildings and roads that are severely heated in the sunshine (Parlow, 2011) have little chance of decreasing their pace of life. Additionally, they have no reason to decrease their body

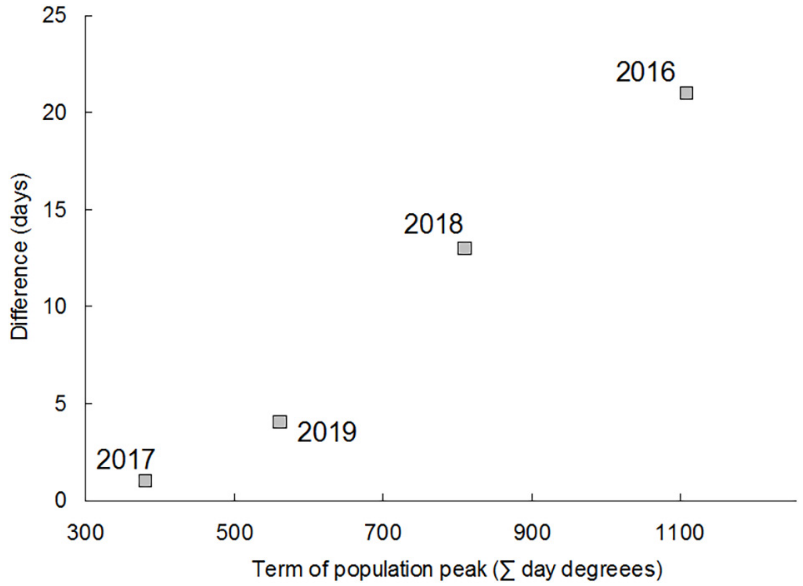

FIGURE 2 | Difference (days) between the term of the population peak in the city center and the term of the population peak in the outskirts of Prague plotted against the thermal time (day degrees) when the maximum abundances were observed (average of the thermal time value in the city center and the outskirts).

temperatures because the ambient temperature remains mostly below the upper temperature threshold of 30°C (Honek et al., 2018a). The temperature maxima in the city center exceeded this limit by 14 times (2016) to 29 times (2019), in most cases (64%) by less than 2°C.

An increase in body temperature above ambient temperature is realized by basking in the sunshine (Sloggett and Honek, 2012; Sloggett, 2021). The capacity to increase body temperature also exists in *H. axyridis* (Michie et al., 2010), but evidence of thermoregulation under natural conditions is indirect. This evidence consists of the geographic variation of the ratio of dark and light color forms that changes in parallel with climate (Dobzhansky, 1924; Komai, 1956). The mechanisms affecting morph distribution include differences in thermoregulation, assortative mating and winter mortality of color morphs (Osawa and Nishida, 1992; Su et al., 2009). This geographic variability is important in some parts of the native area of *H. axyridis*; however, in recently colonized regions of Europe, this phenomenon cannot be reliably demonstrated, as a significant geographic or temporal variation in proportional representation of dark and light forms was not established (Honek et al., 2020a), and thus, the effect of thermoregulation on the life cycle of *H. axyridis*

TABLE 1 | Timing of the peak density of *Harmonia axyridis* in calendar time (Julian days since the release of hibernacula set at 20 April), thermal time calculated using thermal constants of *H. axyridis* as the number of day degrees (dd) above the 10.6°C threshold (Thermal time HA), and thermal time calculated using thermal constants for *E. tiliae* as the number of day degrees (dd) above the 5.0°C thermal threshold (Thermal time ET).

		N	Calendar time Julian days	Thermal time HA dd	Thermal time ET dd
2016	Center	4	131 ± 18.3	1, 126 ± 187.6	2, 084 ± 143.3
	Outskirts	7	152 ± 8.7	1, 092 ± 67.2	2, 056 ± 42.8
			$P_t = 0.028$	$P_t = 0.663$	$P_t = 0.820$
2017	Center	6	63 ± 3.7	423 ± 44.3	1, 037 ± 26.9
	Outskirts	7	64 ± 4.3	341 ± 45.2	845 ± 25.7
			$P_t = 0.557$	$P_t = 0.007$	$P_{MW} = 0.006$
2018	Center	6	86 ± 2.9	807 ± 37.4	1, 571 ± 47.1
	Outskirts	7	99 ± 6.1	815 ± 88.9	1, 570 ± 47.1
			$P_{MW} = 0.002$	$P_{MW} = 0.534$	$P_{MW} = 0.160$
2019	Center	6	76 ± 9.3	603 ± 96.8	1, 303 ± 146.4
	Outskirts	8	80 ± 10.1	522 ± 87.7	1, 124 ± 146.0
			$P_{MW} = 0.491$	$P_{MW} = 0.345$	$P_{MW} = 0.042$

N—number of populations used in the study. P—the significance of differences calculated using the t-test (Pt) or the Mann-Whitney test (PMW).

cannot be reliably demonstrated. Thus thermoregulation likely does not significantly alter the effect of ambient temperature on the population dynamics of *H. axyridis* in the city center and its outskirts.

Ultimate Cause of Variation in the Timing of the Seasonal Peak of Abundance

In *Tilia,* the change in abundance of adult *H. axyridis* parallels that of the abundance of its prey, the monophagous anholocyclic aphid *Eucallipterus tiliae* (Honek et al., 2019a). Aphid abundance estimated in a period of 10–20 days around the peak of *H. axyridis* abundance (Alois Honek and Jiri Skuhrovec, unpublished) exceeded the threshold density of 1 aphid per leaf area necessary for the start of *H. axyridis* oviposition (Honek et al., 2020b). The dependence of *H. axyridis* on its prey determines the early (2017 and 2019) or late (2016 and 2018) timing of the population peaks in particular years. The annual variation in the timing of the peak abundance of *E. tiliae* is well known (Dixon and Barlow, 1979; Dahlsten et al., 1999), likely determined by the size of the overwintering population. The population peak occurs early in the vegetative season if hibernating eggs are abundant; if not, the population peak occurs late in the season (Dixon and Barlow, 1979). As a consequence, the population peaks of *E. tiliae* in the city center and the outskirts in particular years occurred approximately in the same period of the season. The ultimate cause of the timing of the *H. axyridis* population peak is the population dynamics of its prey, *E. tiliae*, which does not depend on the thermal conditions of the season.

Proximate Cause of the Difference in Timing of the Population Peaks in the City Center and the Outskirts

The magnitude of the difference between the *H. axyridis* population peaks in the city center and the outskirts increases with the time that has elapsed since the beginning of the season. The difference in the timing of the population peak levels after the recalculation of the calendar time to thermal time, which passes faster in warm city centers than in colder outskirts. The question remains whether the main factor of the origin of this difference is the temperature effect on the development of the aphid population or, alternatively, is the main factor directly the effect on the predator population? Using our data, this question cannot be reliably resolved, but the assumption of a direct effect on *H. axyridis* populations appears more probable. The effect on *H. axyridis* is significant because temperatures are below the maximum temperature for development, which is approximately 30°C (Auad et al., 2014; Barahona-Segovia et al., 2016) and in this range ambient temperature affects the rate of development of *H. axyridis* populations positively. Temperature also affects the rate of development of aphid populations, but in a different way than in *H. axyridis*. This is because the development threshold for aphid populations is 5°C (Honek and Kocourek, 1990; Honek, 1996), and temperature maximum is then $\leq$ 25°C (Dixon et al., 2009). The temperature in summer often exceed the temperature maxima for aphids, and in this range, does not influence the rate of development of *E. tiliae* positively. It can therefore be assumed that the main reason for the difference in population peak timing is the effect of temperature on populations of *H. axyridis* and not the effect of temperature on the population development of prey.

Significance of the Flexibility of Timing of the *H. axyridis* Life Cycle

This 4-year study enabled us to determine the causes of annual and local variability in the timing of the population peak of adult *H. axyridis*. In particular years, the peak occurred early or late in the season depending on variations in prey abundance. The results corroborated the hypothesis that differences in mesoclimate affect the pace of life of *H. axyridis* and supported the predictions (i) that the increased temperature of the city center, compared to that in the outskirts, advanced the onset of the population peak and (ii) that this difference will level off if population development is plotted against thermal time. Minor differences in the timing of the population peak were due to differences in the mesoclimate in the Prague city center and the outskirts, and the magnitude of the difference increased with the length of time that had elapsed since the beginning of the season. The flexibility of the timing of population development contributes to the success of *H. axyridis* invasion into the non-native area of Central Europe because it enables the effective utilization of prey resources whose location varies in space and time.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

AH and JS designed the experiments and collected data. ZM coordinate all work. AH and PZ analyzed the data and performed the statistical analyses. All authors wrote and edited the manuscript.

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Floral Resources Enhance Fecundity, but Not Flight Activity, in a Specialized Aphid Predator, *Hippodamia convergens* (Coleoptera: Coccinellidae)

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Adult aphid predators disperse across the landscape seasonally in search of prey aggregations that are patchily distributed and temporally variable. However, flight is energetically costly and consumes resources that could be invested in reproduction. *Hippodamia convergens* is an important aphid predator in North American cereal crops and other agricultural systems. Consumption of floral resources can enhance adult survival during periods of low prey availability and may improve reproductive success. We tested how an omnivorous adult diet containing floral resources (diluted honey and pulverized bee pollen) interacts with body size to influence reproduction and flight behavior compared to a prey-only diet. Two sizes of beetles were produced by controlling larval access to food—3 h daily access produced small beetles; *ad libitum* access produced large beetles with faster development. Reproductive performance was tracked for 18 days, and female flight activity was assayed via 3 h bouts of tethered flight. Diet composition and body size interacted to influence preoviposition period, with large females in prey-only treatments delaying oviposition the longest. The omnivorous adult diet improved 18-day fecundity relative to a prey-only diet, but egg fertility was unaffected. Adult size affected oviposition pattern, with small beetles laying smaller, but more numerous, clutches. Females flew up to 7 km in 6 h, but neither body size nor adult diet influenced flight distance, suggesting that all diet treatments generated energy reserves sufficient to power flights of short duration. However, pre-reproductive females flew > 60% further than they did post-reproduction, likely due to the energetic costs of oviposition. Thus, access to pollen and nectar increased reproductive success and altered oviposition patterns in *H. convergens*, indicating the importance of floral resources in the agricultural landscape to conservation of this predator and its biological control services.

Keywords: biological control, life history, energetic trade-offs, insect predators, agricultural ecology, omnivory

INTRODUCTION

The convergent lady beetle, *Hippodamia convergens* Guerin-Meneville, is a generalist insect predator that provides biological control services to agriculture throughout North America. It is primarily a predator of aphids, which adults require for reproduction (Hagen, 1962; Michaud and Qureshi, 2006), but it also preys upon the immature stages of many other agricultural pests as both larva and adult (Hodek, 1996; Michaud, 2018). It has long been associated with agricultural crops in North America, where it has proven adaptable to frequent disturbance, low plant diversity, and other characteristics of industrial agriculture (Hagen, 1962; Sloggett and Majerus, 2000). Predacious coccinellids, in general, appear well adapted to agroecosystems and often persist there at higher densities than in adjacent natural areas (Hagen, 1962; Lamb et al., 2019).

In agriculturally intensive regions of North America, large fields host successions of annual monocultures seasonally, their planting dates dictated by each crop's thermal and hydrological requirements. At landscape scale, this results in an agricultural mosaic of resource patches for arthropod herbivores and their predators that is both spatially variable and temporally dynamic across growing seasons (Bianchi et al., 2009). On the High Plains, adult *H. convergens* track aphid populations across the landscape (e.g., Prasifka et al., 2004), moving predictably between crops such as wheat and alfalfa, where overwintered beetles produce a spring generation, to summer crops such as corn and sorghum, where additional generations occur, contingent on the availability of aphids (Rice and Wilde, 1988; Nechols and Harvey, 1998; Michaud and Qureshi, 2006). They are also able to survive extended periods of prey deprivation by consuming various plant-derived resources, including tender green foliage, pollen, and nectar of both floral and extra-floral origin (Hodek, 1996; Michaud and Qureshi, 2005, 2006; Mercer et al., 2020). However, utilization of floral resources in an agricultural landscape also carries a risk of exposure to systemic insecticides now widely employed as seed treatments (Gontijo et al., 2014; Moscardini et al., 2015; Bredeson and Lundgren, 2018) in addition to foliar applications (He et al., 2012).

Supplemental foods of plant origin not only support the survival of predaceous coccinellids in the absence of animal prey (Hodek, 1996; Hatt and Osawa, 2019) they are often necessary for the beetles to achieve maximal fitness (Lundgren, 2009; Michaud, 2018; Stowe et al., 2021). Consequently, these resources are also consumed during periods of prey abundance (Berkvens et al., 2010; Hodek and Evans, 2012; Choate and Lundgren, 2013; Ugine et al., 2019), and may be selectively consumed when particular nutrients are lacking in the diet (Schuldiner-Harpaz and Coll, 2017). The inclusion of plant resources in the diet of aphidiophagous coccinellids can facilitate earlier onset of oviposition (Hatt and Osawa, 2019) and enhance egg fertility (De Clercq et al., 2005; de D'Ávila et al., 2017). Dietary self-selection of foods rich in specific macronutrients is well documented in many insects (Jensen et al., 2012), both under normal conditions (Jones and Raubenheimer, 2001; Mayntz et al., 2005), and in response to physiological deficits (Raubenheimer and Jones, 2006; Raubenheimer et al., 2007). Ratios of macronutrients in the diet can influence insect reproduction and flight behavior by affecting the availability of metabolic precursors and titers of metabolites and hormones, as exemplified by anautogenic mosquitos (Attardo et al., 2005; Hansen et al., 2005). In the case of facultatively omnivorous predators such as *H. convergens*, the consumption of plant resources could influence energetic trade-offs between dispersal and reproduction. An improved understanding of any such tradeoffs, and the role of plant-derived nutrients in mediating them, could provide key insights for conserving the biological control services provided by these predators in agricultural landscapes.

Flight is a behavior critical to the survival and fitness of *H. convergens* because adult beetles must track populations of their ephemeral aphid prey across large spatial scales in the agricultural landscape in order to achieve reproductive success (Hagen, 1962; Wissinger, 1997; Michaud, 2012). Dispersal by flight can be critical to biological control efficacy as insect predators move among crops, and flight behavior will have a significant impact on energy budgets (Bonte et al., 2012). Dietary effects on flight capacity could potentially affect the range of beetle movement, and thus the ability of beetle populations to track and control pest populations. As yet, relatively few studies have examined *H. convergens* flight behavior (but see Rankin and Rankin, 1980; Davis and Kirkland, 1982; Abdel-Wahab et al., 2017), and none have addressed the potential of plant-derived resources to affect flight capacity. The present study was designed to examine how access to floral resources might affect flight capacity and distance flown by *H. convergens* females, and how the energy expended in flight might exact a cost in terms of reproductive performance. Because body size is a key morphological trait that affects both wing loading (Byrne et al., 1988), and potential fecundity (Vargas et al., 2013), we created two size classes of adult beetles by controlling larval access to food. We hypothesized that both larger adult size and access to floral resources would benefit reproductive success, and enable beetles to fly longer distances when compared to smaller beetles, and those with access only to prey. We also hypothesized a negative correlation between flight distance and reproductive success, a trade-off that should be more pronounced in smaller beetles with fewer energy reserves. Finally, we used structural equation modeling to explore direct and indirect relationships between adult body size, diet composition, and reproductive and flight metrics to determine if early energy expenditures influence later performance.

MATERIALS AND METHODS

Insect Colonies

Adult *Hippodamia convergens* beetles (ca. 120 individuals) were collected from wheat fields at the Agricultural Research Center-Hays, in Hays, Kansas (38°51′32.1″N 99°20′07.7″W) in early June, 2020. Beetles were placed in a 1 L glass mason jar covered with muslin netting and held in a climate-controlled growth chamber set to 24 ± 1°C, 50–60% RH, and a 16:8 (L:D) photoperiod. Wax paper strips were placed in the jar as harborage, water was provided on a cotton wick, and 10–20 mg of frozen eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) were added to the jar every 48 h. Because *H. convergens* are often parasitized by *Dinocampus coccinellae* Shrank (Hymenoptera: Braconidae), the colony was examined daily for the first week to remove all emerging parasitoid larvae before they could pupate or energy as wasps. Under these conditions, beetles remain in reproductive diapause and can be held for extended periods (Michaud and Qureshi, 2006).

A colony of greenbug, *Schizaphis graminum* Rondani, was established from material collected from the same wheat field as the beetles. Aphid colonies were reared in metal trays of wheat seedlings in a mixture of soil, vermiculite, and peat moss (1/1/1). Trays were germinated in a greenhouse and then were moved to growth chambers set to 22 ± 1°C, 50–60% RH, and a 16:8 (L:D) photoperiod. Plants were infested at the 2-leaf stage by spreading infested wheat cuttings over fresh trays of seedlings.

Experimental Beetles

For the experiment, parental females (n = 29) were isolated in plastic Petri dishes (5.5 cm diam) and held in a climate-controlled growth chamber under the same conditions as the stock colony. Females were provisioned with greenbugs *ad libitum* to induce oviposition and the fertility of each female was confirmed by verifying the eclosion of eggs in her initial clutches. Once a sufficient number of fertile females were ovipositing (n = 29), a single day's oviposition was collected from each for incubation. were collected from eclosing clutches on 18 September, 2020. Neonate larvae were permitted to consume their chorions and disperse from their natal egg mass before they were collected and isolated in Petri dishes (as above). Neonates observed cannibalizing conspecific eggs or other larvae were excluded from the experiment, as cannibalism can alter developmental times (Michaud and Grant, 2004; Bayoumy and Michaud, 2015). Each larva was labeled according to maternal lineage (n = 29) to ensure that similar numbers of offspring from each lineage were assigned to each treatment (ca. n = 24 couples, per treatment), and that sibling adults were never paired together within treatments.

Larvae were reared on frozen eggs of *E. kuehniella* and provided water on a small cube of sponge, both refreshed daily. Larvae assigned to the "small adult" treatment were permitted access to food for only 3 h daily (although water remained continuously available), whereas those assigned to the "large adult" treatment had *ad libitum* access to food. Upon pupation, each petri dish was cleaned of any remaining food to prevent consumption by newly emerged adults and ensure an accurate fresh weight at emergence could be obtained. Adult beetles were all sexed and weighed (within 24 h of emergence) on an analytical balance (Mettler Toledo, AG285, Columbus, OH). Non-sibling beetles from the same treatment were then paired in ventilated snap cap vials (5 cm diam × 9 cm ht).

Both small adult pairs (n = 48) and large adult pairs (n = 48) were further divided into two groups each, one receiving an omnivorous diet, the other receiving a prey-only diet, both provided *ad libitum*. The omnivorous diet consisted of greenbugs, provided on excised leaves of their host plant, frozen *E. kuehniella* eggs, pulverized bee pollen, dilute honey on a sponge cube (1:2 honey:water), and water on another sponge cube, all provided *ad libitum*. In contrast, the prey-only diet lacked the floral resources and consisted of only greenbugs on wheat foliage, frozen *E. kuehniella* eggs, and water on a sponge cube, all provided *ad libitum*. Both greenbugs and water were refreshed daily, whereas *E. kuehniella* eggs, bee pollen, and diluted honey were refreshed every third day. Initially, all beetle pairs were maintained in reproductive diapause for 18 days by withholding the greenbug component of the diet, as female *H. convergens* reared on *E. kuehniella* eggs do not become gravid until provisioned with aphids *ad libitum* for 3–4 days (Michaud and Qureshi, 2006). This was done so that we could flight-test all females on a similar time frame both before and after a period of reproduction.

Experimental Design

The experiment was organized in a 2 × 2 factorial design with adult size (large vs. small) and adult diet (omnivorous vs. prey-only) as independent factors, yielding four treatments: (1) small, omnivorous couples (SO, n = 25), (2) small prey-only couples (SP, n = 24), (3) large omnivorous couples (LO, n = 25), and (4) large prey-only couples (LP, n = 25).

Flight Assays

Flight mills (n = 16) were constructed, modified from the design of Attisano et al. (2015), which allowed measurement of total flight distance when connected to recording software. A small spot of magnetic primer (Rust-oleum magnetic primer) was applied to the distal portion of the right elytra of each beetle using a wooden toothpick. After the paint dried (ca. 24 h), each female was attached to a small neodymium magnet on the end of a flight mill arm, where she flew at will in a 10 cm diameter circle in a climate controlled growth chamber set to 24 ± 1°C and 50–60% RH. The flight mill arm rotated freely around a frictionless magnetic bearing and distance flown was measured by an infrared light sensor that tracked each revolution of the arm. Data was recorded continuously from the sensors using an Arduino MEGA 2560 REV3 single board microcontroller and terminal program (CoolTerm). For the initial flight test, each female was placed on a mill for a total of 3 h, then returned to her dish. Following 18 days of oviposition, tallied consecutively from production of her first clutch, each female was flown for another 3 h period.

Reproduction

Following the addition of greenbugs to the diets, females in both diet treatments were monitored daily for oviposition. Egg clusters, usually laid on the opaque lids of the vials, were collected and held until eclosion under the same physical conditions as the stock colony. The number of eggs in each clutch (daily oviposition) was counted before and after eclosion to obtain total fecundity and egg fertility for each female. This continued for a period of 18 days, a period that is usually sufficient to detect any dietary effects on reproductive success (Michaud, 2005). Females were held together with males from pair formation until the end of the experiment to maximize female fertility. Females that did not oviposit within 30 days after access to greenbugs were concluded to be non-reproductive and completed their second flight test. These non-reproductive females (n = 2) were excluded from reproductive analysis, but included in flight observations, as they expressed the lowest reproductive effort.

Statistical Analysis

All data passed tests for equality of variance (Levene's test) and normality (Shapiro-Wilk test) and were analyzed by two-way ANOVA, followed by a Bonferroni test to separate means. Preoviposition period was tallied for each female as the no. days from first provision of greenbugs to the first day of oviposition, 18-days fecundity as the total number of eggs laid in 18 days, counted from first oviposition day, and egg fertility as the percentage of all eggs hatching. Oviposition days were tallied as the total number of days, out of 18, on which at least one egg was laid. Flight distance was tallied as the total number of revolutions completed on the flight mill, multiplied by the circumference of the mill arm. We also used linear regression to test for relationships between pairs of continuous response variables, and a paired *t*-test to compare distances flown by beetles pre- and post-oviposition.

Structural equation modeling (SEM) was employed to characterize direct and indirect relationships between independent variables (body size, adult diet) and components of female fitness (preoviposition period, no. oviposition days, fecundity, egg fertility) and flight performance (pre- and post-ovipositional flight distances). Categorical experimental predictors were coded for adult size as 0 (small) and 1 (large), and diet treatment as 0 (prey-only) and 1 (omnivorous). Maximum likelihood methods were used for model selection with goodness of fit assessed by Akaike's Information Criterion (AIC) in a step-wise process. Model selection began using the complete hypothesized model with influence expected between all biologically feasible pathways, then removing non-significant relationships at each step, beginning with the highest *p*-value. We also used tests of directed separation to identify factors whose addition (or reintroduction) could improve model fit. The AIC value of each successive model was consulted at each step of the model selection process to assess goodness of fit and models with AIC decreases > 2 were considered better fit. Models with AIC value changes < 2 were differentiated by parsimony (Burnham and Anderson, 2002; Grace, 2006). R version 4.0.3 was used to conduct all analyses in the piecewise SEM package (Lefcheck, 2016).

RESULTS

Larvae restricted to 3 h daily food access were smaller at adult emergence than those reared with *ad libitum* access to food; females weighed less (16.4 ± 0.3 mg vs. 21.7 ± 0.3 mg, $F = 119.7$; df = 1, 97; $P < 0.001$), and developed more slowly (22.5 ± 0.2 day vs. 19.0 ± 0.1 day; $F = 218.7$; df = 1, 97; $P < 0.001$).

Both adult size ($F = 11.01$, df = 1, 95; $P = 0.001$) and adult diet ($F = 4.95$; df = 1, 95; $P = 0.028$) affected how long females took to begin oviposition after they began receiving greenbugs, but these factors did not interact significantly ($F = 2.47$; df = 1, 95; $P = 0.119$). Females in the LP treatment took longer to begin oviposition than females in other treatments, the latter being not significantly different from each other (**Figure 1**). Similarly, adult size ($F = 5.64$; df = 1, 95; $P = 0.020$) and adult diet ($F = 5.43$; df = 1, 95; $P = 0.022$) affected the number of oviposition days in the 18 days observation period, again without any significant interaction between factors ($F = 0.001$; df = 1, 95; $P = 0.981$). Females in the SO treatment laid clutches on more days than did females in the LP treatment, with other treatments not significantly different (**Figure 2**). The 18-days fecundity of females was not affected by adult size ($F = 3.30$; df = 1, 95; $P = 0.073$), but strongly affected by adult diet ($F = 13.06$; df = 1, 95; $P < 0.001$), without any interaction between these factors ($F = 0.91$; df = 1, 95; $P = 0.343$). Fecundity was higher in the SO treatment than in either the SP or LP treatments, with no other differences among treatments significant (**Figure 3**). Egg fertility averaged 62.8 ± 2.9% (mean ± SE) and was unaffected by body size ($F = 2.19$; df = 1, 95; $P = 0.142$) or adult diet ($F = 0.16$; df = 1, 95; $P = 0.687$).

Neither adult body size ($F = 0.001$; df = 1, 95; $P = 0.975$) nor adult diet ($F = 0.17$; df = 1, 95; $P = 0.685$), affected the initial (pre-reproductive) flight distance of females, although the interaction term between these factors was marginally significant ($F = 3.72$; df = 1, 95; $P = 0.057$). Similarly, the distance flown by females after an 18-days reproductive period was unaffected by adult body size ($F = 2.12$, df = 1, 95; $P = 0.815$) or adult diet ($F = 0.06$; df = 1, 95; $P = 0.148$. Female flight activity varied greatly, with total distance flown ranging from < 5 m to > 7 km. Beetles flew significantly further on their first test than on their second ($t = 5.93$; df = 1, 98; $P < 0.001$; **Figure 4**). Regression analysis revealed that preoviposition period was negatively correlated with both number of oviposition days ($F = 45.35$; df = 1, 97; $P < 0.0001$; $r^2 = 0.32$) and 18-days fecundity ($F = 29.38$; df = 1, 97; $P < 0.0001$; $r^2 = 0.02$). However, fecundity was not correlated with flight distance, whether pre-reproductive ($F = 0.11$, df = 1, 97; $P = 0.741$) or post-reproductive ($F = 0.19$, df = 1, 97; $P = 0.277$).

Structural equation modeling indicated no significant effect of pre-reproductive flight on subsequent reproductive success or flight behavior (**Figure 5**, Fisher's $C = 10.5$, df = 22, $P = 0.98$). Adult size and diet both influenced the preoviposition period, with large adult size delaying onset of oviposition ($\beta_A = 0.32$, $P = 0.001$) and the omnivorous diet accelerating it ($\beta_D = -0.21$, $P = 0.020$). Omnivory also had a direct positive effect on fecundity ($\beta_H = 0.16$, $P = 0.004$) in addition to its indirect positive effects on fecundity via effects on preoviposition period ($\beta_C = -0.54$, $P < 0.001$) and number of oviposition days, the latter having a direct positive effect on fecundity ($\beta_F = 0.80$, $P < 0.001$). Delayed onset of oviposition was also associated with greater post-reproductive flight distance ($\beta_B = 0.27$, $P = 0.008$). Fertility and fecundity were positively correlated with one another ($\beta_G = 0.29$, $P = 0.002$) and fertility was positively correlated with post-reproductive flight distance ($\beta_E = 0.21$, $P = 0.04$).

DISCUSSION

The omnivorous adult diet resulted in greater female fecundity than did the prey-only diet, supporting our initial hypothesis,

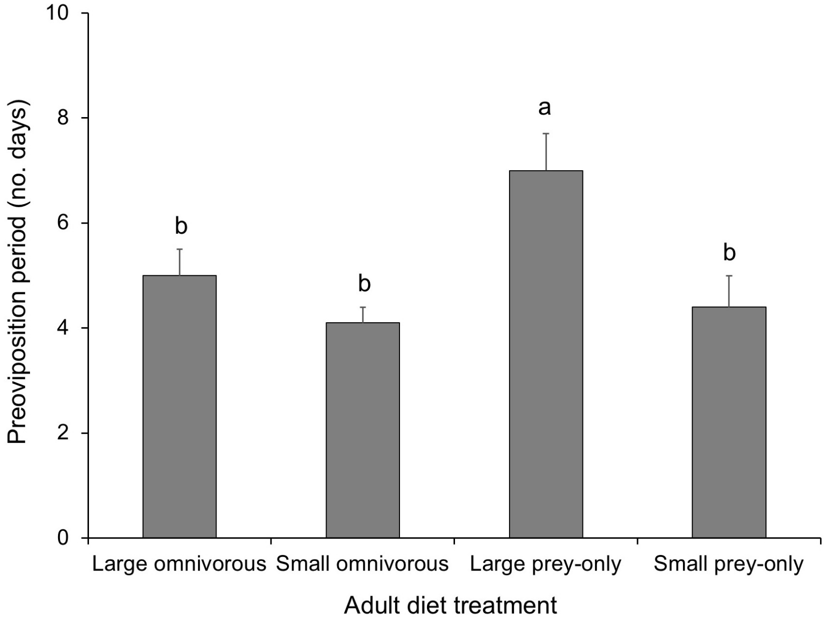

FIGURE 1 | Mean (+ SE) preoviposition periods, of large (*ad libitum* larval food access) and small (3 h daily larval food access) *Hippodamia convergens* pairs subjected to one of two different adult diet treatments: omnivorous (pollen, honey, *Schizaphis graminum* and *Ephestia kuehniella* eggs), or prey-only (*S. graminum* and *E. kuehniella* eggs). Treatments bearing the same letter were not significantly different (Tukey's test, α = 0.05).

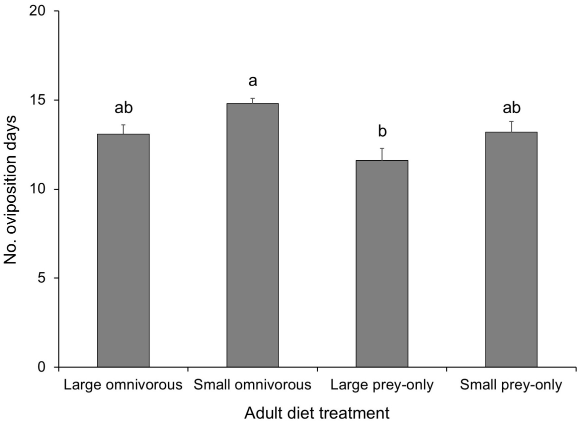

FIGURE 2 | Mean (+ SE) number of oviposition days (no. days on which clutches were laid out of 18 days of observation) for large (*ad libitum* larval food access) and small (3 h daily larval food access) *Hippodamia convergens* pairs subjected to one of two different adult diet treatments: omnivorous (pollen, honey, *Schizaphis graminum* and *Ephestia kuehniella* eggs), or prey-only (*S. graminum* and *E. kuehniella* eggs). Treatments bearing the same letter were not significantly different (Tukey's test, α = 0.05).

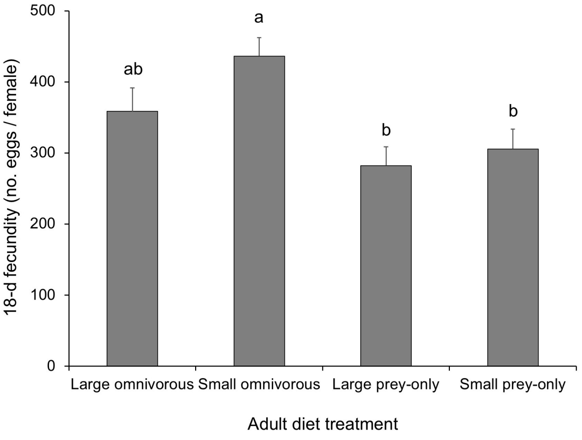

FIGURE 3 | Mean (+ SE) 18-days fecundities of large (*ad libitum* larval food access) and small (3 h daily larval food access) *Hippodamia convergens* pairs subjected to one of two different adult diet treatments: omnivorous (pollen, honey, *Schizaphis graminum*, and *Ephestia kuehniella* eggs), or prey-only (*S. graminum* and *E. kuehniella* eggs). Treatments bearing the same letter were not significantly different (Tukey's test, α = 0.05).

and confirming that floral resources complement the nutrition provided by prey, and are essential to maximal reproductive success in this species. Previous studies have similarly concluded that plant-derived foods generally enhance the performance of predatory coccinellids relative to prey-only diets (Lundgren, 2009), including *H. convergens* (Stowe et al., 2021). Supplemental (plant-derived) foods have long been recognized for their importance to coccinellid survival during periods of prey scarcity (Hodek, 1996; Michaud and Qureshi, 2005), but may also provide phytosterols, key requirements for development and reproduction that insects are unable to synthesize endogenously (Levinson, 1962; Behmer and Nes, 2003; Pilorget et al., 2010). Furthermore, feeding on carbohydrates can increase lipid reserves, which can improve insect longevity and overwintering

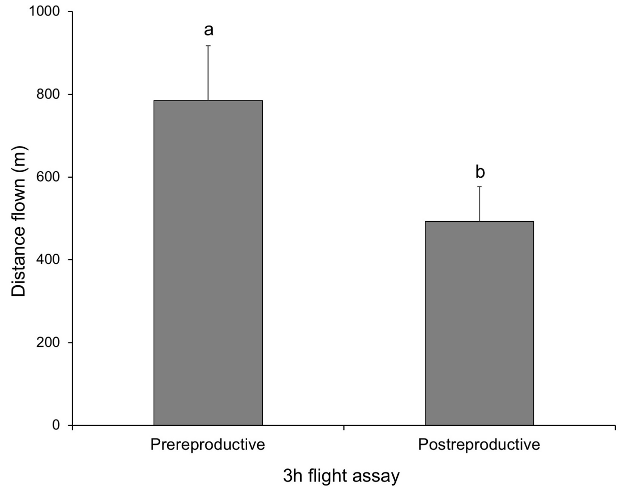

FIGURE 4 | Mean (+ SE) distances flown by *Hippodamia convergens* females (all treatments pooled) during 3 h bouts of tethered flight prior to oviposition (Prereproductive), and after 18 days of oviposition (Postreproductive). Values were significantly different (paired *t*-test, $P < 0.001$).

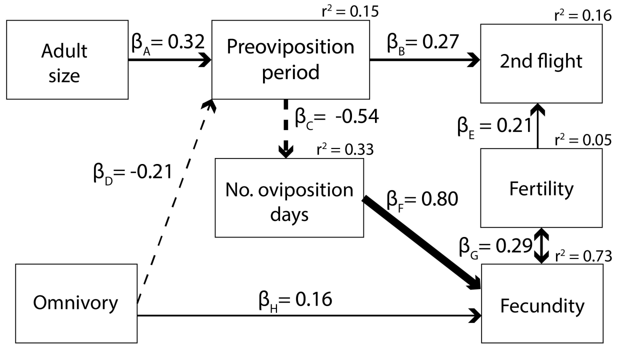

FIGURE 5 | Structural equation model (SEM) of the effects of adult body size (small vs. large) and adult diet, either prey-only or prey plus floral resources (omnivory) on the reproduction and flight performance of female *Hippodamia convergens*. Solid arrows represent positive causal relationships and dashed arrows, negative causal relationships. Fecundity is the number of eggs laid per female in 18 days of oviposition. Preoviposition period is the number of days from addition of *Schizaphis graminum* to the diet to first oviposition. No. oviposition days is the number of days on which clutches were laid in the 18-day observation period. Second flight distance was the distance flown by females after the 18-days oviposition period while tethered on a flight mill for 3 h. Fisher's C = 10.49, df = 22, $P = 0.98$.

survival (He and Sigsgaard, 2019), and can be rapidly oxidized to provide energy for flight (Toprak, 2020; Wang et al., 2020). The benefits of omnivory likely occur independent of prey diversity in the diet, as animal and plant-derived foods typically supply key macronutrients in differing ratios (Raubenheimer and Jones, 2006; Raubenheimer et al., 2007). Macronutrient ratios have been shown to affect growth, development, and survival in phytophagous coccinellids (Wang et al., 2018) and other insect herbivores (Behmer, 2009), and arthropod predators often forage selectively to balance their intake of protein, lipids and carbohydrates (Mayntz et al., 2005; Jensen et al., 2012). For example, a prey-only diet will be high in protein, which can reduce net energy intake due to digestive costs, and alter activity levels (e.g., Koemel et al., 2019).

Our hypothesis that large body size would result in greater female fecundity was not supported, seemingly contradicting previous work that examined the effect of *H. convergens* body size on fecundity over longer time frames (Vargas et al., 2012a,b), although plant resources were not provided in those experiments. Adult size only had a marginal effect on fecundity within the 18-days observation period, and in the opposite direction of that expected, being highest for small females. This result may reflect fewer days of oviposition by LO and LP pairs within the period of observation, and/or delayed onset of oviposition in LP pairs. Oviposition days were strongly correlated with fecundity in the SEM model, and previous work has shown that daily fecundity peaks later in large *H. convergens* than in smaller ones (Vargas et al., 2012b), so a longer period of observation could reveal different body size effects. Fertility was unaffected by either diet or adult size, contradicting our hypothesis that larger body size would benefit fertility. Once again, this result likely reflects the limited time frame of our observations. Maternal body size has no discernable effect on *H. convergens* egg fertility early in reproductive life, but its effects become more pronounced with advancing age, as fertility declines faster in both large and small females than in those of intermediate size (Vargas et al., 2012b).

Our hypothesis that access to floral resources would positively affect reproductive success was supported. Large beetles fed a prey-only diet experienced delayed onset of oviposition relative to those receiving the omnivorous diet, suggesting that nutrients provided by floral resources enabled beetles to begin maturing eggs sooner when body size was large. However, the large beetles produced in this experiment are likely uncommon in nature, where food-limitation is the norm for most larvae at some point in development. If reproductive traits have evolved to be adaptive for the average body size, a large body size may impose some costs. Large individuals may have higher maintenance requirements than small ones, such that the soma of large beetles may (initially) take precedence over the gonads when certain key nutrients become limiting, as likely occurred in the prey-only treatment.

Despite the fact that body size and adult diet affected beetle reproductive parameters, neither treatment generated any significant variation in the distance flown by females on flight mills, either in pre- or post-reproductive tests. In large part, this was due to immense variation among females in total flight activity, which ranged from < 5 m to > 7 km. Rankin and Rankin (1980) similarly reported large variation in distances flown by tethered *H. convergens* beetles, with 90% of individuals stopping after 30 min, while the remaining 10% were still flying after 12 h. We infer that *H. convergens* populations consist of individuals that vary greatly in intrinsic flight propensity, and that additional variation was probably contributed by differences in physiological state, despite our best efforts to standardize beetle age and rearing conditions. Maes et al. (2014) showed that body size was positively correlated with greater flight distance in both *Harmonia axyridis* Pallas and *Cryptolaemus montrouzieri*

Mulsant, although not in *Adalia bipunctata* (L.), so it is possible our observation period was not sufficiently long to resolve a body size effect. Although nectar can be an essential source of energy fueling flight in many insects, including Diptera (Dunn et al., 2020), Hymenoptera (Suarez et al., 2005), and Lepidoptera (Su et al., 2021), most of the energy obtained by nectar feeding is stored as lipids in the fat body, the "coordination center" of lipid metabolism in insects (Toprak et al., 2020), and lipids are the energy currency of sustained flight in most insects (Canavoso et al., 2003; Van der Horst et al., 2003). Our results indicate that *H. convergens* adults do not require access to sugar to fuel flight, but likely rely upon stored lipids, as do most other insects (Arrese and Soulages, 2010; Toprak, 2020). Some of these lipid reserves may be accumulated during larval development, and in the case of some Coleoptera, almost entirely (e.g., Tussey et al., 2018; Dvoracek et al., 2020), thus rendering adults less dependent on floral resources. This could be adaptive for *H. convergens*, given that adult beetles may need to make long distance migratory flights across landscapes where flowers are unavailable, such as after emergence from hibernation or aestivation (Hagen, 1962).

Dispersal by flight is energetically costly and is expected to reduce the energy available for subsequent foraging behavior and/or reproduction (Stearns, 1992). The SEM results revealed direct, but weak, relationships between post-reproductive flight distance and both length of the preoviposition period and egg fertility, implying that both delayed onset of oviposition and higher egg fertility were associated with greater flight activity in gravid females. More significantly, the shorter distances flown by females post-reproduction suggest that egg maturation depleted the energy reserves available for flight. This result stands in contrast with the findings of Stewart and Gaylor (1994) for the tarnished plant bug, *Lygus lineolaris*; older bugs flew greater distances than preproductive ones. Our adult diet treatments did not impact energy reserves sufficiently to affect flight activity within a 3 h period, nor did this period of flight deplete energy reserves sufficiently to negatively affect subsequent reproductive capacity. These results are consistent with a long-range dispersal capability in this species, with much longer periods of flight required before energetic demands become significant.

Apparent trade-offs between flight ability (or energetic expenditure on flight) and reproductive effort have been reported in many insects (Zera and Harshman, 2001). For example, Guerra and Pollack (2007) showed that flight ability was negatively correlated with male courtship singing in the cricket *Gryllus texensis*. Roff (1986) compared 22 species of wing-dimorphic insects and found that brachypterous female morphs were more fecund and had earlier onset of reproduction than macropterous morphs, and Zera and Denno (1997) showed that flight capability carries reproductive costs for males as well as females. In the present study, we found that post-reproductive flight distance was positively correlated with preoviposition period in the SEM model, the latter being negatively correlated with number of oviposition days, and thus with fecundity, results that are also consistent with a trade-off between reproductive effort and energy expenditure on flight.

The quality of the natal patch is known to have strong carryover effects on dispersal capacity. For example, high emigration rates of the backswimmer *Notonecta undulata* are associated with development in patches of high quality (Baines and McCauley, 2018). Because the quality of the developmental environment has the potential to shape adult phenotypes, it can have cascading effects on population dynamics and community structure that extend to landscape scales (Van Allen and Rudolf, 2013, 2016). Carryover effects of natal patch quality on body size and life history parameters will have significant implications for *H. convergens* population dynamics and the predation services this species provides in the agricultural landscape, as successive generations colonize different crops that vary in the quality of resources they provide. Thus, the provision of floral resources either within, or adjacent to, crop fields will help sustain the vigor of *H. convergens* populations, and the efficacy of their biological control services. These services extend beyond control of aphids to the consumption of many other pests in their early life stages, even though the latter may not support beetle reproduction (Michaud, 2018).

Our results illustrate the importance of floral resources to the fitness of arthropod predators that provide biological control services as they migrate across the agricultural landscape. Broadleaf plants have distinct, usually brief, flowering seasons, so a diversity of species that vary in flowering phenology will be required to ensure these resources are available over extended periods (Bianchi et al., 2006). Many cultural practices have the potential to support, or disadvantage, populations of coccinellids and other generalist predators in the landscape and warrant careful assessment of their ecological impacts. For example, overzealous control of all broadleaf plants on roadsides and marginal lands constitutes an unnecessary reduction of potential floral diversity in the service of cultural expectations, with no direct impact on crop production. Also concerning is the increasingly wide-spread use of insecticidal seed treatments. This practice not only impacts predators negatively via prey deprivation during crop establishment, but creates various routes of direct and indirect exposure for beneficial species, with both lethal and sublethal consequences (Seagraves and Lundgren, 2012; Gontijo et al., 2014; Moscardini et al., 2015; Bredeson and Lundgren, 2018, 2019). In contrast, farmer tolerance of flowering broadleaf plants in marginal and uncultivated areas, and the planting of cover crops as an alternative to sterile, "chem-fallow" periods that create starvation conditions for all arthropods, represent feasible approaches to supplement valuable floral resources for predator populations as they move among crops in the agricultural landscape.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

HS and JPM conceived and designed the experiment with conceptual contributions from TK. HS conducted the experiments and compiled the data. HS and TK analyzed the data and constructed the SEM. HS and JPM wrote the manuscript in consultation with TK. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Invasion of the Asian Ladybird *Harmonia axyridis* (Pallas, 1773) in the European Part of Russia

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The natural range of the Asian ladybird *Harmonia axyridis* (Pallas, 1773) covers the territories of China, Mongolia, Korea, Japan, and Russia (Far East, Southern Siberia, and Altai). Since the early 1980's, the global invasion of *H*. *axyridis* began, first on the territory of the United States, from where the Asian ladybird spread to South America, Africa, and Europe. By 2016, this species occupied all of Western Europe with the exception of its northern and southern regions. Penetration of *H. axyridis* on the territory of the Russian Federation (Kaliningrad) was discovered in 2010, and since 2011, the species has spread to the Black Sea coast. This report presents our data on the distribution of *Harmonia axyridis* in central Russia, from its western borders, to the Volga River. The occupation of this huge territory by this species occurred mainly in the last 3 years—2018–2020. The rate of movement of the species on the territory of the European part of Russia is about 200 km/year.

Keywords: Asian ladybug, *Harmonia axyridis*, invasion, propagation speed, European part of Russia

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INTRODUCTION

The natural range of the Asian ladybird *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) covers the following territories: North-Eastern and Central China, Mongolia, Korea, Japan, Eastern Kazakhstan, and in Russia—the Far East, southern Siberia, Tyva, and Altai (Orlova-Bienkowskaja et al., 2015; Blekhman and Goryacheva, 2017; Andrianov et al., 2018). In the early 1980's, the global invasion of *H. axyridis* began first in the United States (Koch et al., 2006), from where the Asian ladybird spread to South America, Africa, and Europe where it mixed with populations used in biological protection of greenhouse crops that escaped into the open (Lombaert et al., 2010; Roy et al., 2016). In Europe, invasive populations of *H. axyridis* were discovered in the early 2000's (Adriaens et al., 2003), and by 2016, this species occupied all of Western Europe except for its northernmost and southernmost regions (Roy et al., 2016).

In the European part of Russia, the occurrence of *H. axyridis* was observed from 2004 to 2006 in the territory of the Belgorod Region (Binkovskaya, 2004; Orlova-Bienkowskaja, 2013). In 2010, mass reproduction of the species was recorded by one of the authors in Kaliningrad (Zakharov et al., 2011). The presence and reproduction of *H. axyridis* have been observed since 2011 on the Black Sea coast of the Caucasus (Orlova-Bienkowskaja, 2013; Ukrainsky, 2013) and since 2012–2013 in the Krasnodar Territory (Korotyaev, 2015a,b). During the same period, there were reports of *H. axyridis* finds in Ukraine [since 2007—(Nekrasova and Tytar, 2009; Verizhnikova and Shylova, 2013)], in Belarus [since 2011—(Kruglova, 2015; Kruglova and Sinchuk, 2017)], and in Latvia [since 2009—(Barševskis, 2009)].

This report presents the authors' data on the distribution of *H. axyridis* in central Russia, from its western borders to the Volga River. The discussed species captured this huge territory mainly in the last 3 years—2018–2020.

MATERIALS AND METHODS

The presence of *H. axyridis* was recorded during the implementation of special collections of coccinellids or when collecting other insects. Beetles *H. axyridis* were collected at the stages of imago, pupae, and last instar larvae. Collections were carried out on various shrubs and trees, in cities—mainly on lime trees (*Tilia* sp.), as well as during the autumn flight and in wintering areas. Methods of collecting beetles and keeping them in the laboratory are described in Andrianov et al. (2018). In the collections of *H. axyridis*, where morphs were counted, three phenotypes were distinguished: *succinea* (yellow or red with a different number of black spots), *spectabilis*, and *conspicua* (both are black, with four and two red spots, respectively) and *axyridis* (black with many red/yellow spots). Among the *succinea* morphs, the proportion of individuals without spots was taken into account (see **Table 2**).

RESULTS AND DISCUSSION

Information about the materials collected by the authors of this paper and the collections of other researchers is presented in **Table 1** and the map (**Figure 1**). It can be seen that *H. axyridis*

TABLE 1 | Collections of *Harmonia axyridis* in 2018–2020 years in the European part of Russia.

Collection sites (westerly to easterly)	Geographical coordinates (lat/long)	Dates of collections	Collections—solitary/mass	Population state	Collector or publication
Kaliningrad	54.71 / 20.51	08.2019	Mass	B	I.A. Zakharov
Kursk region	52.31 / 35.38	10.06.2019	Solitary	B	Ruchin et al., 2020
Oryol	52.96 / 36.08	08.10.2018	Solitary	A	Sazhnev et al., 2020
Kaluga	54.53 / 36.17	10.2018	Solitary	C	Ruchin et al., 2020
Moscow, Gagarin square	55.71 / 37.58	14.10.2020	Mass	C	I.A. Zakharov
Moscow, South district	55.61 / 37.68	09.2020	Mass	B	I.A. Zakharov
Moscow, Zhulebino district	55.69 / 37.84	06-08.2020	Mass	B	D.A. Romanov
Sochi, Lazarevskoe	43.91 /39.33	06.2019	Mass	B	D.A. Romanov
Rostov-on-Don	47.22 / 39.72	10.2019	Mass	A	D.E. Romanov
Ryazan region	53.59 / 40.03	10.2019	Mass	D	I.I. Goryacheva
Ryazan region	54.21 / 40.98	6–13.10.2020	Solitary	A,D	A.B. Ruchin
Ryazan region	54.07 / 41.45	6–13.10.2020	Solitary	A,D	A.B. Ruchin
Tambov region	52.49 / 42.80	24.08.2019	Solitary	A	Ruchin et al., 2020
Republic of Mordovia, Pushta	54.72 / 43.23	10.2019 10.2020	Mass	D	A.B. Ruchin
Saransk	54.18 / 45.18	10.2019 10.2020	Mass	A,C,D	A.B. Ruchin E.A. Lobachev A.V. Meshcheryakova
Volgograd region	49.55 / 45.07	15.09.2018	Solitary	A	Ruchin et al., 2020
Saratov	51.52 / 45.98	06.09.2019	Mass	A	Ruchin et al., 2020
Penza region	52.78 / 46.80	10.07.2018	Mass	A	Ruchin et al., 2020
Republic of Chuvashia	54.51 / 46.35	23.08.–25.10.2019	Solitary	A	Egorov et al., 2019
Republic of Chuvashia	55.00 / 46.42	14.10.2020	Solitary	A	L.V. Egorov
Cheboksary	56.08 / 47.08	14.10.2020	Solitary	A	N.V. Borisova
Cheboksary	56.08 / 47.15	23.08.–25.10.2019	Solitary	A	Egorov et al., 2019
Republic of Chuvashia	54.51 / 47.22	27.06.2020	Solitary	A	L.V. Egorov
Ulyanovsk region	53.08 / 47.38	25.07.2019	Solitary	A	Ruchin et al., 2020
Ulyanovsk,	54.18 / 48.07	3.10.2020	Solitary	A	Kichigin, 2020
Astrakhan	46.35 / 48.03	10.04.2019	Solitary	A	Ruchin et al., 2020
Kazan	55.85 / 49.07	30.09.2019	Solitary	B	Ruchin et al., 2020
Samara region	53.74 / 49.45	27.06.2020	Solitary	A	D.S. Pasynkova
Samara region	53.46/ 49.84	29.08.2019	Solitary	A	Egorov et al., 2019
Republic of Udmurtia	58.13 / 52.67	24.09.2019	Solitary	A	Sazhnev et al., 2020

Population state: A – active, only imago, B – reproduction (presence of pupae and larvae), C – winter flight, D – wintering; solitary/mass - single find / mass collection.

FIGURE 1 | *Harmonia axyridis* distribution in the European part of Russia (2018–2020).

TABLE 2 | Constitution of *Harmonia axyridis* populations, number and percent of morphs.

Collection sites	Dates of collections	Total	Conspicua	Spectabilis	Axyridis	Melanic		Succinea, total	succinea without spots	
						Total	Percent (95%c.i.)		Total	Percent (95%c.i.)
Kaliningrad	08.2019	164	1	1	-	2	1.2 (0.02–4.34)	162	6	3.7 (1.35–7.79)
Moscow, South district	09.2020	84	1	10	-	11	13.0 (/6.72–22.22)	73	1	1.2 (/0.03–6.46)
Moscow, Zhulebino district	06-08.2020	169	1	6	-	7	4.1 (1.68–8.35)	162	2	1.2 (0.14–4.21)
Sochi	06.2019	107	-	6	-	6	5.6 (2.09–11.81)	101	25	23.4 (15.73–32.53)
Rostov-on-Don	10.2019	69	4	5	-	9	13.0 (6.14–23.32)	60	7	10.1 (4.18–19.79)
Republic of Mordovia, Pushta	10.2020	268	5	16	-	21	7.8 (4.92–11.73)	247	9	3.4 (1.55–6.28)
Saransk	10.2019 10.2020	206	3	13	1	17	8.3 (4.88–12.88)	189	3	1.5 (0.30–4.20)

95%c.i., 95% "exact" confidence interval.

occupied a huge territory—from the western borders of the Russian Federation to the Volga.

Almost exclusively the morphs found commonly in Europe—*succinea*, *spectabilis*, *conspicua*—were present in all the collections, with *f. succinea* dominating. The West Siberian morph *axyridis* was found only once—in Saransk (one individual). The least number of melanics (*spectabilis, conspicua*) was in Kaliningrad. The proportion of beetles of the *succinea* morph without spots was the greatest in Sochi in comparison with other populations (**Table 2**). This suggests that the settlement of the Black Sea coast of the Caucasus occurred not only from the west, as in other regions, but also from the south, from Asia Minor.

TABLE 3 | The ratio of three species of coccinellids in collections in Moscow, Zhulebino district (end of June – August 2020).

Species	Number of larvae	Number of pupae	Total
Harmonia axyridis	21	148	169
Adalia bipunctata	56	457	513
Adalia decempunctata	14	91	105

It is known that in the new territories occupied by *H. axyridis*, this species successfully competes with the local Coccinellidae, as a result of which the number of the latter decreases (Brown and Roy, 2018). In two geographical locations, the authors calculated the ratio of *H. axyridis* and local species of the genus *Adalia* [*Adalia bipunctata* (Linnaeus, 1758) and *Adalia decempunctata* (Linnaeus, 1758)], which occur on the same plants with aphid colonies and reproduce at the same time. In Kaliningrad (August 2019), where *H. axyridis* were collected, *Adalia* ladybirds were not found at all, although in previous years, before the appearance of *H. axyridis* here, *Adalia* findings were numerous (observations of IZ). The same was noted by one of the authors (IZ, unpublished) in Munich (Germany), where in 2015, with a significant number of found *H. axyridis*, *Adalia* ladybirds were not found. In Moscow, where mass reproduction of *H. axyridis* was observed for the first time in 2020, *Adalia* ladybirds still predominate among coccinellids (**Table 3**). These data should be supplemented in subsequent years.

The rate of progress of the species was estimated from the findings in Kaliningrad (2010), the first breeding colony in Moscow [2015—(Zakharov, 2015)], and in Saratov [2019—(Sazhnev et al., 2020)]. Taking into account the distance from Kaliningrad to Moscow (1,100 km) and from Kaliningrad to Saratov (1,750 km), the propagation speed is about 200 km/year. At this speed, beetles can spread both naturally (Jeffries et al., 2013) and using passing transport. The rate of spread calculated here is the same as that calculated in other parts of Europe (Brown et al., 2011).

Given the wide adaptability of *H. axyridis* to various environmental and climatic conditions, one can expect the spread of this species in the coming years to the north, at least to St. Petersburg (in 2019, *H. axyridis* was not there yet) and to the east, to the Urals.

If *H. axyridis* spreads to the east beyond the Urals, it will be possible to observe an interesting natural experiment, when the populations of two subspecies of *H. axyridis*, which clearly differ in morphological features [color and pattern on the elytra—(Blekhman and Goryacheva, 2017)] and in mitotypes—variants of mitochondrial DNA (Zakharov et al., 2011), unite in the Tomsk—Novosibirsk area (the western edge of the native range). Given the rate of spread of *H. axyridis*, one can expect that nature will stage this population-genetic experiment in the next 10 years.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

IZ conceived the project, designed methodology, and led the writing of the manuscript. IZ, AR, LE, DR, and AS contributed to ladybird collection and identification. All authors contributed critically to the drafts and gave final approval for publication.

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Development of the European Ladybirds Smartphone Application: A Tool for Citizen Science

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Wildlife observations submitted by volunteers through citizen science initiatives are increasingly used within research and policy. Ladybirds are popular and charismatic insects, with most species being relatively easy to identify from photographs. Therefore, they are considered an appropriate taxonomic group for engaging people through citizen science initiatives to contribute long-term and large-scale datasets for use in many different contexts. Building on the strengths of a mass participation citizen science survey on ladybirds in the United Kingdom, we have developed a mobile application for ladybird recording and identification across Europe. The main aims of the application are to: (1) compile distribution data for ladybird species throughout Europe, and use this to assess changes in distribution over time; (2) connect and engage people in nature and increase awareness about the diversity and ecological importance of ladybirds. In developing the application we first constructed a database including ladybird species from the United Kingdom, Czech Republic, Slovakia, Italy, Belgium, and Portugal with associated information on relevant morphological features (e.g., size, main color, pronotum pattern) to inform identification. Additionally, the species were assessed on the basis of probability of occurrence within each country which enables users to reduce the number of species to only those with relevance to the location of the recorder. This is amongst the first collaborative citizen science approaches aimed at involving participants across Europe in recording a group of insects. In the near future, we aim to expand the use of the application to all countries in Europe.

Keywords: coccinellids, community science, species distribution, large-scale, smartphone mobile app

INTRODUCTION

Citizen science, otherwise known as public participation in scientific research or community science, can be a powerful tool to obtain data on insect diversity and abundance across larger spatial scales whilst simultaneously promoting education and awareness about insects (e.g., Eitzel et al., 2017; Gardiner and Roy, 2021). Indeed, considering reports of global insect declines (e.g., Hallmann et al., 2017; Cardoso and Leather, 2019; Wagner et al., 2021), enhancing citizen science or community science, especially in areas where academic or professional infrastructure is lacking, was one of the immediate actions proposed by scientists when formulating a roadmap for insect conservation and recovery (Harvey et al., 2020). Cardoso et al. (2011) recognized the role of citizen science as part of the solution for the scarce and underfunded science on invertebrates, including the declining number of insect taxonomists. There are various typologies of citizen science and viewpoints on what is or is not citizen science (Haklay et al., 2021). Here, we focus on volunteers and their contributions to field-based observations in a coordinated effort involving professional scientists (Bonney, 1996).

Ladybirds (Coleoptera: Coccinellidae) are popular and charismatic insects. More than 6,000–7,000 species have been described worldwide (Seago et al., 2011). The brightly colored forewing patterns of ladybirds enable relatively easy identification to species-level from photographs for most species (e.g., Jouveau et al., 2018). As such, ladybirds from the more conspicuous groups (Coccinellinae, Epilachninae, Chilocorinae) are highly suitable for engaging people within citizen science projects (e.g., Gardiner et al., 2012).

Ladybirds are very diverse in terms of biology and ecology. Different species can be found in many terrestrial habitats (e.g., deciduous and coniferous forest, meadows and marshes, heathlands, croplands) where they exploit a range of prey species (e.g., aphids, coccids, whiteflies, mites, fungi) (for more detail, see Hodek et al., 2012). Some species display specific life history traits such as habitat and/or dietary specialization, myrmecophily and aggregation behavior which make them attractive to naturalists and fascinating to the public (Adriaens et al., 2015a). Although ladybirds are considered one of the most well-studied groups of insects, there are many knowledge gaps and scientific discoveries still to be made about them. Recently there was a first report of parthenogenesis in ladybirds (Magro et al., 2020); a group that, until recently, was considered to have only sexual reproduction.

While Jarić et al. (2020) reported that the charisma of a species (*sensu* Ducarme et al., 2013) highly affects the public's approach toward it, Groom et al. (2021) suggest that the target species/group needs to also evoke emotion or connection in the participants and that this is probably more important than simply the degree to which the species is considered charismatic. It is known that in zoo gardens, an animal's beauty and (large) size are generally more important to visitors than is conservation status (Frynta et al., 2013). In citizen science the connection to volunteers (i.e., citizens, scientists, biodiversity managers, policy-makers, local authorities, industry, schools, and other participants) can be positively (e.g., Sequeira et al., 2014) or negatively emotive (e.g., Gallo and Waitt, 2011; Palmer et al., 2017; Porter et al., 2019). European ladybirds are deeply rooted in popular culture and are undoubtedly regarded as both emotive and charismatic. They may invoke positive or negative emotions (e.g., Shipley and Bixler, 2017). As an example, the arrival of the invasive alien harlequin ladybird, *Harmonia axyridis* (Pallas) has been the motivation for increasing participation in established ladybird recording schemes worldwide (e.g., Roy and Brown, 2015; Grez et al., 2016; Hiller and Haelewaters, 2019). This species was introduced to many countries, has spread rapidly across the globe and is considered a threat to native biodiversity (Roy et al., 2012, 2016). *Harmonia axyridis* is regarded both positively, for being charismatic and for the ecosystem services it provides (Riddick, 2017), and negatively, adversely affecting other species of aphid natural enemy through competition and intra-guild predation (Roy et al., 2012; Brown et al., 2015a; Kenis et al., 2017; Masetti et al., 2018; Zaviezo et al., 2019). Additionally it is considered a nuisance to humans in some contexts.

Mobile applications (apps) have played a key role in facilitating the participation of volunteers in citizen science projects (August et al., 2015; Chandler et al., 2017). Mobile apps can help record the location of a species and allow for fast and easy submission of records without data loss when connectivity is good (Teacher et al., 2013). Nowadays, there are numerous mobile apps available for biological recording, with a general or specific taxonomic scope, and some with advanced built-in image recognition tools to facilitate species identification (e.g., iNaturalist, Observation.org or Pl@ntNet). Most Europe-wide biodiversity projects capture occurrence information (opportunistic data) to support atlas projects (e.g., butterflies—Settele et al., 2008; birds—Herrando et al., 2019; mammals—Mitchell-Jones et al., 1999), and increasingly use records collected via citizen science initiatives using mobile apps. Recent statistical developments provide methods for assessing biodiversity trends and indicators (e.g., Termaat et al., 2019) at large scales, using opportunistic data collected through citizen science (Isaac et al., 2014). Structured, abundance sampling schemes and partnerships also provide biodiversity assessments at the European scale. Schemes for butterflies are the most established for insects and provide European indicators for measuring progress toward biodiversity targets (e.g., Sustainable Development Goal: *Life on Land*; van Swaay et al., 2019). It is widely recognized that there is potential to increase the contribution of citizen science to enhance our understanding of trends across many insect groups (Gardiner and Roy, 2021).

Here, we provide an overview, including the rationale, of the development of a dedicated mobile app for European ladybird identification and recording. Our main aims in designing the application were to engage diverse audiences in sharing their sightings of ladybirds whilst providing information on ladybird ecology and identification to participants, in order to improve appreciation of the diversity and value of these popular beetles. We provide technical specifications of the application, including mechanisms for data sharing, data quality and record validation. Finally, we outline potential approaches for using this recording tool to enhance trans-national collaboration on ladybird mapping in Europe.

MONITORING OF EUROPEAN LADYBIRDS IN SEMINAL NATIONAL PROJECTS

Various European countries have operated ladybird recording schemes of different kinds for several decades. Some of the schemes are run at a national level whilst others are regional. Such schemes, as detailed below, were key in developing the ideas for a pan-European approach to ladybird recording.

United Kingdom

There has been a national recording scheme for ladybirds in the United Kingdom since 1971. This evolved into the United Kingdom Ladybird Survey[1] in 2005, one of the first online wildlife recording schemes (Brown et al., 2008). Together these have engaged tens of thousands of people with recording the distribution and ecology of ladybirds in the United Kingdom, contributing over 229,000 verified records. The United Kingdom Ladybird Survey launched the smartphone app iRecord Ladybirds in 2013. The current European Ladybird App builds on that model. The substantial recording effort in the United Kingdom has greatly increased our understanding of the distribution and ecology of native and introduced ladybird species. In particular, verified records of *H. axyridis* have allowed the unusual opportunity of highly detailed mapping of the spread of a new invasive species (Brown et al., 2008, 2018).

Czech Republic

Monitoring of ladybirds in the Czech Republic has been partially covered by the work of the Nature Conservation Agency of the Czech Republic (AOPK CR), which monitors all living organisms using the BioLog and iNaturalist applications. There is also monitoring of *H. axyridis* on the popular BioLib server. In 2019, the *NAJDI.JE*[2] platform was launched for monitoring invasive invertebrates in the Czech Republic using citizen science, including *H. axyridis* overwintering sites. Additionally the databases of taxonomists Ivo Kovář and Oldřich Nedvěd contain a large amount of data, obtained by determining records for a very large base of amateur entomologists within the Czech Entomological Society.

No targeted ladybird monitoring project has yet been created in the Czech Republic, so the European Ladybird App will enable widespread recording of ladybirds in the country.

Belgium

In Belgium, a large-scale citizen science ladybird mapping project was launched in 1999. Skilled volunteer recorders collected standardized data on ladybird occurrence and ecology using a standard recording form (Adriaens et al., 2008). The aim was to use these data to publish distribution atlases (Branquart et al., 1999; Adriaens and Maes, 2004) and a Red List (Adriaens et al., 2015a). The project was supported by the Research Centre for Nature, Forests and Wood in the Walloon Region and the Institute for Nature Conservation (INBO) in Flanders. Observations were underpinned with pictures or other means of verification (e.g., based on recorder experience). Updated distribution maps were published regularly and people were actively stimulated to fill gaps through dedicated searches in insufficiently sampled grid squares. The project engaged about 800 volunteers actively submitting records. The data gathered were openly published on the Global Biodiversity Information Facility in 2012 (Adriaens et al., 2021). Since 2008, after the launch of the online reporting portal[3] and its mobile phone applications by the conservation NGO Natuurpunt (cf. Swinnen et al., 2018), paper recording was replaced by online recording which greatly increased the number of ladybird observations, the speed of record submission, the number of volunteers as well as the spatial resolution and quality of ladybird records. As this platform is well established for recording, the European Ladybird App is anticipated to be used less used in Belgium by the established naturalist community, though it might still appeal to people wanting to increase their ladybird identification skills, wanting to contribute to European ladybird mapping specifically, as well as to a crowd outside the classical recording community. As several recording tools generate useful ladybird data, the focus should be on openly publishing data and sharing them through the application of data standards and interoperability (Adriaens et al., 2015b; Groom et al., 2016).

Slovakia

Haviar (2007) contributed much to the knowledge of occurrence, distribution and ecology of ladybirds in Slovakia. Related short-term monitoring and research activities in this country targeted communities of arboricolous ladybirds in forest and urban habitats (Panigaj et al., 2014; Viglášová et al., 2017; Holecová et al., 2018) and the year-round dynamics of *H. axyridis* in Scots pine forest habitats (Zach et al., 2020). The citizen science project Ladybirds of Slovakia (2015–2017)[4] focused on ladybird data collection from volunteers via Facebook.

Portugal

In the Azores (Portugal), since 2019, a project that allows citizens to assist in the monitoring of ladybirds is available.[5] Under this project, face-to-face activities are organized for schools of all levels of education and institutions with children in need. Scientists and students undertake field work and training to collect and record ladybirds. Under this project, two important outputs were already produced; an updated checklist of the ladybird species of Portugal, including the Azores and Madeira archipelagos (Soares et al., 2021) and a biodiversity database of ladybirds (Coleoptera: Coccinellidae) of the Azores archipelago (in prep.). There is no equivalent project for mainland Portugal, so the European Ladybird App will enable widespread recording of ladybirds in the country.

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[1] https://www.coleoptera.org.uk/coccinellidae/home/

[2] https://www.najdije.cz

[3] www.waarnemingen.be

[4] https://www.facebook.com/lienkyslovenska

[5] www.joaninhasdosacores.com

Italy

Many short-term monitoring and research activities informed the knowledge of habitats and distribution of ladybirds in Italy. Many local papers updated the distribution of *Harmonia axyridis* at regional level and data from citizen science, i.e., iNaturalist or local bioblitzes (see Menchetti et al., 2016), have been used in scientific literature. There is no global recording scheme for ladybirds in Italy, so the European Ladybird App will enable widespread recording of ladybirds in the country (**Figure 1**).

COLLABORATIVELY DEVELOPING THE EUROPEAN DATABASE OF LADYBIRDS

In developing this application we first set up a team with expertise in coccinellids from six countries (United Kingdom, Czech Republic, Slovakia, Italy, Belgium, and Portugal) (**Figure 1**). We collaboratively prepared a database of ladybird species established within each country. We excluded small, so-called inconspicuous ladybird species, which are known to be difficult to identify (e.g., the genus *Scymnus*). Currently, 72 taxa are documented in the database, including various color forms for some species (e.g., *H. axyridis* has six color forms across the countries) (**Figure 1C**). For each species, we included the following information to support identification (**Figure 1B**): (1) morphology (size, elytral coloration, pattern on pronotum, presence of melanic forms, information about spot fusions, coloration of legs, number of spots), (2) ecology (prey, habitat, plants and overwintering habitat), and (3) three photos of adults (where available). To maximize accessibility to volunteers across the participating countries, we translated all the text into the official languages for each country.

Technical Specifications

This open-source mobile application (*link*)[6] was developed using standard web-based technologies (HTML, CSS, JavaScript). This way we were able to reuse many supporting open-source libraries and tools which helped to streamline the development process. The app is essentially a responsive website that is designed to work well on small mobile devices. As a website it couldn't be directly installed onto users' smartphone devices therefore it was wrapped with a Cordova container. Traditionally, mobile applications are re-written for each mobile platform but packaging the code in such a way allows reuse of the same codebase for multiple platforms. Such an app is called a Hybrid and it can be deployed to both iOS and Android app stores.

The app has species names and other related content translated and managed by multiple collaborators from different countries (**Figure 1G**). The species datasheet is extensive and currently holds over 160 columns with species-related information. We are hosting the datasheet online on OneDrive cloud which essentially is our simple but flexible real-time content management system. We have agreed on a custom column header formatting schema which allowed us to write a command-line script to transform the columns into a JSON-formatted file which is then pulled into the app programmatically. In such a way, collaborators from multiple countries can update the content in parallel and the updates can then be imported into the app with little effort and few errors.

Using a Microsoft Excel spreadsheet for translations for multiple countries wasn't very practical, so we use the Transifex web platform for managing species and app interface translations. At the time of writing, it is free for open-source projects such as this and allows us to pull the translations into the app.

The app is linked to an open-source Indicia database (warehouse)[7] where the records are stored. It has a lot of tooling to streamline the verification process and to export and exchange data with other systems. Indicia has extensive online documentation[8] of all aspects of installing and maintaining a data warehouse to support data capture projects, including mobile application approaches such as European Ladybird App (**Figure 1**). The system architecture, data model and coding conventions allow any developers to contribute to the open source development of the software through the project Github repository.[9]

Data Quality and Validation

The use of Indicia for data storage gives a flexible solution for data sharing agreements and allowing species records to be linked to the same user accounts from multiple connected mobile applications and websites. One of the sites our mobile app is linked to is iRecord[10] which is used in the United Kingdom for sharing wildlife observations, supports multiple recording apps such as this and has a big community of recorders and verifiers.

iRecord incorporates verification rules developed by national recording schemes for a desktop application (the National Biodiversity Network Record Cleaner). Validation is assisted by automated record checks which highlight records outside the known spatial or temporal range of the species, and records of species which are difficult to identify. If records are highlighted by Record Cleaner the observer receives a message, with the option of editing the record. The expert verification of records on iRecord applies a two tier approach. Verifiers choose from a set of validation categories that they can apply to a record. A record is *accepted* as meeting the standard required for inclusion by the recording scheme or project in question when a verifier accepts it as *correct* on the basis of the submitted photo or is *considered correct* when the verifier has not seen photo/s or specimen/s (or cannot be absolutely sure of the identification from submitted photo/s) but has a high degree of confidence that the record is likely to be correct, based on difficulty of ID, date, location and recorder skills/experience etc. Other categories include *unable to verify*, *incorrect*, *unconfirmed* or *plausible*.

Citizen Focus

The application requires participants to create a record of the ladybird they observe and so, it is necessary for them to identify

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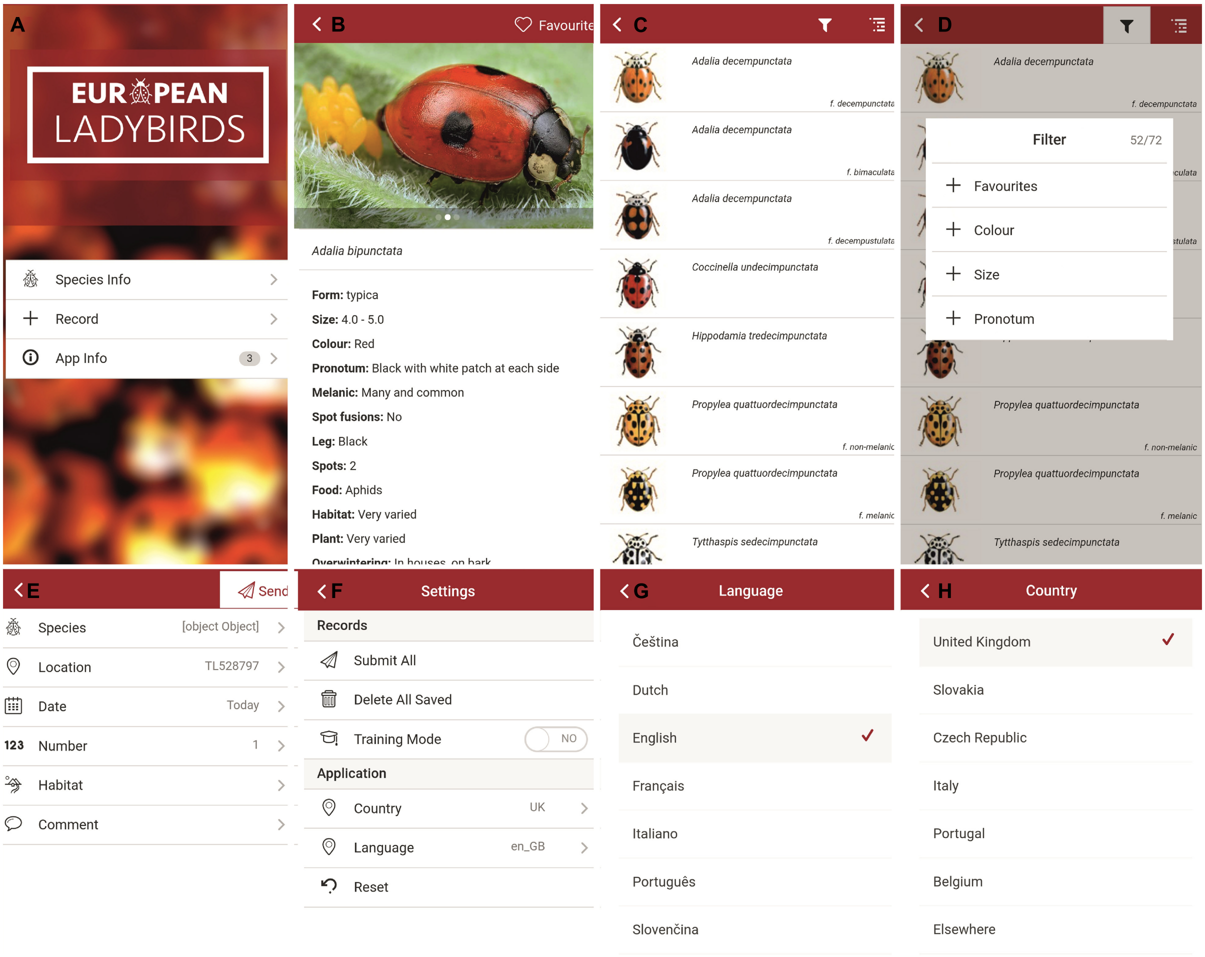
[6] https://github.com/NERC-CEH/leu-app

[7] http://www.indicia.org.uk/

[8] https://indicia-docs.readthedocs.io/en/latest/index.html

[9] https://github.com/Indicia-Team/

[10] https://www.brc.ac.uk/irecord/



Adalia bipunctata

Form: typica
Size: 4.0 - 5.0
Colour: Red
Pronotum: Black with white patch at each side
Melanic: Many and common
Spot fusions: No
Leg: Black
Spots: 2
Food: Aphids
Habitat: Very varied
Plant: Very varied
Overwintering: In houses, on bark

Species [object Object]
Location TL528797
Date Today
Number 1
Habitat
Comment

Records
Submit All
Delete All Saved
Training Mode NO
Application
Country UK
Language en_GB
Reset

FIGURE 1 | Screenshots from the European Ladybirds smartphone application (app) used to record ladybird species, with *Adalia bipunctata* used as an example throughout. Records submitted are checked iRecord: **(A)** opening screen; **(B)** ladybird guide: providing identification information for the species selected, in this case *Adalia bipunctata*; **(C)** ladybird guide: first screen, showing a list of species available to record, ranked by likelihood of occurrence; **(D)** ladybird guide: first screen, showing a list of species available to record, filtered by four traits of the ladybird; **(E)** adding a record: main recording screen once a species has been selected; **(F)** information: screen provides background information about the app; **(G)** information: screen provides for language selection; **(H)** information: screen provides for country selection.

the species, which will later be verified by an expert. Additionally, the following information is requested: the coordinates of the location (usually derived from mobile phone GPS), date, number of individuals, habitat where the individual was found, and a photo (to enable identification). To increase the ease of use we selected the three most specific morphological features (main color, size, and pronotum pattern) and coupled these with country-based ranking of the relative occurrence (based on the total number of grid cells with observations of the species) for each species. Such filters (**Figure 1D**) enable the users to select from a reduced number of likely species when recording, thus helping to reduce misidentifications.

Potential Use of Data

Information collated through the European Ladybird App will be openly available to address many potential questions but we focus on three main areas: (1) Understanding the ecology and distribution of ladybird species; (2) Informing ladybird conservation; (3) Education and engagement in biological recording and entomology. In a preliminary release of the European Ladybird App, we received over 1,600 records of 26 species between January 2019 and May 2021, mostly from GB and Ireland but also from other places in Europe. About 75% of these records have been accepted as accurate, indicating the high potential of this app for citizen science.

This initiative is the first step of a collaborative approach involving the recording of ladybirds through citizen science across Europe[11] (**Supplementary Figure 1**). Currently, the application builds on existing collaborations between six countries that have ladybird recording, but in the long-term we aim to include additional countries and use the data generated to produce a European ladybird distribution atlas. This will also

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[11] https://european-ladybirds.brc.ac.uk/home

require integrating the data from the ladybird app with other available data on ladybird distribution. This can be achieved through an open approach to publishing and sharing data from the app and the promotion of the app with other active mapping projects in Europe. Long-term conservation trends for ladybirds are so far only available for very limited regions (e.g., United Kingdom; Roy and Brown, 2018) or databases (e.g., GBIF—the Global Biodiversity Information Facility) and we aim to use the European data to inform Red List assessments (Maes et al., 2015)[12] Additionally the app has the potential to generate timely data on the arrival of new ladybird species in a country: for example in the United Kingdom, the first records of *Oenopia conglobata* L. were received via similar citizen science tools (Brown et al., 2015b).

Furthermore, ladybirds offer an appealing way of encouraging people to have an interest in entomology; in many ways ladybirds represent an "easy way in" to entomology, which can appear a daunting field. We consider the successful engagement of people to be as important as the ecology and conservation knowledge gained from the records themselves. The engagement benefits come from encouraging an interest in wildlife and the environment, as well as encouraging the next generation of entomologists and biological recorders. Additionally, connecting with nature is known to enhance the well-being of participants (Pritchard et al., 2020).

CONCLUSION

In conclusion, the development of the European Ladybird App highlights the value of collaborative partnerships in citizen science. By working on a shared platform and using infrastructures employed for other similar, but national initiatives, we have been able to cost-effectively develop an approach for people across Europe to engage with studying and recording ladybirds. Future work could focus on how the app is performing in different countries, which species are reported, recording biases etc. and how it can be improved to suit the needs of the recording community. There is an urgent need to gather evidence on the status and trends of insects over large spatial scales to inform conservation action going forward. Providing resources, such as this smartphone application, will play an important part in maximizing the potential of citizen science and increasing access and participation in the field of entomology.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.741854/full#supplementary-material

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[12] https://www.iucn.org/commissions/ssc-groups/invertebrates/ladybird

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Urbanization Negatively Affects the Species Composition of Native Ladybirds in Central Europe

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We evaluated the differences in frequency (percentage of sampling sessions where the species was recorded) and abundance (mean number of individuals per 100 sweeps) of native species of ladybirds (Coleoptera: Coccinellidae) in the center of Prague, Central Europe, and in its outskirts. Between 2016 and 2019, coccinellids were sampled on *Tilia* stands using a standardized sweeping method. Twenty-one species were identified in the total sample of 2,761 adults that were collected in 504 sampling sessions. Species richness was significantly higher in the outskirts than in the center, where seven species were absent and two species were found only once. In contrast, only five species were more abundant in the center than in the outskirts. Communities of native coccinellids were poorer in species, and most species were less abundant in the center than in the outskirts.

Keywords: urban habitats, invasive, ladybirds, species richness, abundance

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INTRODUCTION

The decline in abundance and diversity of insect communities is a current topic of concern to society as a whole (e.g., Hallmann et al., 2017; Sánchez-Bayo and Wyckhuys, 2019). This is because some factors in this process, e.g., climate change and habitat loss, are influenced by human activity (Ceballos et al., 2017; Meyer et al., 2020; Milanovic et al., 2021; Wagner et al., 2021). An important factor threatening insects is urbanization (McGlynn et al., 2019), which increases the area of urban settlements at the expense of agricultural land, forest, and other land uses (Goddard et al., 2010; Jones and Leather, 2012; Rocha et al., 2018; Milanovic et al., 2021). The effect of urbanization has been well-studied on ladybirds (Coleoptera, Coccinellidae) (Honek et al., 2021; Sloggett, 2021). This family includes a number of predatory species feeding mainly on aphids (Hodek et al., 2012). The aphidophagous species of coccinellids are easily identifiable (Jouveau et al., 2018), they can be picked up by sampling methods, which allows their research in the urban environment where they are assembled mainly on trees infested with aphid populations (Honek et al., 2017; Rocha et al., 2018). In addition to the availability of prey, coccinellids species are also affected by the microclimate, the possibility of immigration from source populations living in the city surroundings, and interactions with other species of the guild of aphid predators (Korányi et al., 2021; Sloggett, 2021). Therefore, coccinellids are a suitable subject for study into the effect of urbanization on insects. Covering urban areas with impervious surfaces, such as buildings and paved or tarmac roads, has a negative effect on the quality of habitats: urban green spaces (where coccinellid prey and its host plants can persist) is fragmented into small discontinuous islands (Rocha et al., 2018; Sloggett, 2021). Urbanization of large areas can thus negatively affect the presence and abundance of coccinellids in the area by reducing the availability of breeding sites and increasing the need for migration abilities of coccinellids to enable displacement from undisturbed habitats of the city surroundings to the center or between green islands in urban areas (Sloggett, 2021). Fragmentation of green spaces may cause a shortage of food and extinction or emigration of the local population of coccinellids.

Each city differs in both the size of its urbanized area and the expansion and degree of fragmentation of its urban green spaces (Cepelová et al., 2017; Greyvenstein et al., 2021). To study the effects of these differences, it is necessary to compare coccinellid populations in centers and outskirts for a number of different sized cities. Here, we report the results of a study investigating coccinellid occurrence and abundance in Prague, a city of ∼1 million inhabitants located in the temperate zone of Central Europe. We used the results of a four-year study (2016–2019) of coccinellid communities on lime trees (*Tilia* spp.) carried out in this city, which enabled evaluation of differences in composition and abundance of native species in the outskirts and center. As the known negative impact of urbanization, we hypothesize that in the center of Prague, there will be lower diversity and abundance of coccinellids than in the outskirts.

MATERIALS AND METHODS

Sampling

Between 2016–2019, coccinellids were sampled in two areas, henceforth called "outskirts" and "center," situated at a distance of ∼10 km in the western part of Prague, Central Europe. The difference in urbanization at the outskirts and center areas was quantified as the percentage of the area surrounding the sampling sites (a circle of 500 m radius centered at each sampling site) covered by impervious surfaces (buildings, streets, parking places, etc.) (Honek et al., 2018b). This value was 39 ± 3.0% in the outskirts and 71 ± 5.6% in the center. This was determined using maps available at https://en.mapy.cz. Coccinellids were sampled at 14 localities (henceforth called sites), eight in the outskirts, and six in the center (**Figure 1**). At each site was a group of ≥5 lime trees (*Tilia cordata* Mill. and/or *Tilia platyphyllos* Scop.). Coccinellids were sampled on these groups of lime trees in regular intervals (**Table 1**). Sampling at a particular site and on a particular date is henceforth called a "sampling session." In each sampling session, the coccinellids were collected of all lime trees present at the site. The result of the sampling session was therefore a mixed set of coccinellids from all lime trees growing at the site, henceforth called sample. The total number of samples collected is shown in **Table 2**. Sampling from the canopy up to a height of c. 3 m was standardized by using a standard sweep net (35-cm diameter, 140-cm handle) operated by J.S. (center) and A.H. (outskirts). Sampling (80–200 sweeps at each site) occurred between 08.00 and 18.00 h on sunny and calm days. The coccinellid adults were identified regarding species (for illustration see Nedvěd, 2020) immediately and were released at the site.

Describing Species Composition

Analyses of results from sampling sessions included samples that consisted of ≥1 native coccinellid adult. The presence of coccinellid species was described by two characteristics: (i) the presence/absence of the species in the sample and (ii) the abundance of species, which was the number of individuals recalculated per 100 sweeps. Characteristic (i) was used as a standard to evaluate the differences in species occurrence between outskirts and centers, and characteristic (ii) was used to study the intraspecific differences in population density between the outskirts and centers. The occurrence of a particular species in an area (outskirts or center) was described as the percentage of sampling sessions with a positive characteristic value (i) and its abundance as the average of the characteristic values (ii). Characteristics (i) and (ii) were correlated, and a significant linear relationship was found between the two characteristics (**Figure 2**).

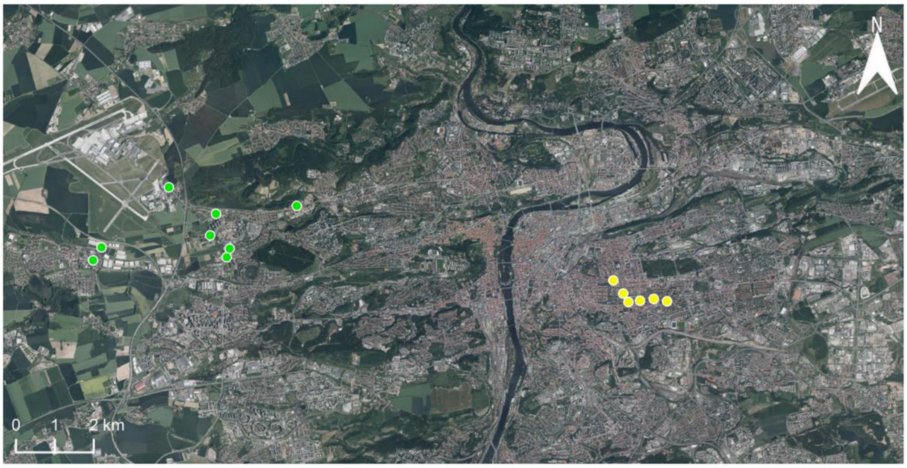

FIGURE 1 | The geographic position of sampling sites in Prague outskirts (8 sites, green dots) and center (6 sites, yellow dots).

TABLE 1 | Dates of sampling in the outskirts and in the center.

	Date of sampling	
Year	**Outskirts**	**Center**
2016	10-May; 20-May; 06-Jun; 21-Jun; 04-Jul; 12-Jul; 22-Jul; 27-Jul; 08-Aug; 22-Aug; 24-Aug; 05-Sep; 21-Sep; 05-Oct; 15-Oct; 27-Oct; 07-Nov	06-May; 11-May; 20-May; 30-May; 07-Jun; 13-Jun; 21-Jun; 28-Jun; 11-Jul; 18-Jul; 25-Jul; 04-Aug; 15-Aug; 24-Aug; 08-Sep; 14-Sep; 27-Sep; 07-Oct; 24-Oct
2017	10-May; 16-May; 23-May; 29-May; 08-Jun; 14-Jun; 21-Jun; 28-Jun; 07-Jul; 17-Jul; 25-Jul; 04-Aug;15-Aug; 23-Aug; 30-Aug; 06-Sep; 11-Sep; 27-Sep; 04-Oct; 17-Oct	25-Apr; 03-May; 10-May; 17-May; 26-May; 05-Jun; 13-Jun; 22-Jun; 30-Jun; 10-Jul; 16-Jul; 21-Jul; 31-Jul; 07-Aug; 14-Aug; 22-Aug; 30-Aug; 08-Sep; 18-Sep; 27-Sep; 13-Oct; 26-Oct
2018	09-May; 18-May; 24-May; 31-May; 06-Jun; 11-Jun; 20-Jun; 26-Jun; 04-Jul; 18-Jul; 26-Jul; 07-Aug; 14-Aug; 27-Aug; 05-Sep; 16-Sep; 17-Sep; 30-Sep; 16-Oct	27-Apr; 04-May; 11-May; 21-May; 28-May; 31-May; 11-Jun; 18-Jun; 27-Jun; 04-Jul; 13-Jul; 20-Jul; 27-Jul; 06-Aug; 16-Aug; 23-Aug; 03-Sep; 20-Sep; 04-Oct; 19-Oct
2019	06-May; 21-May; 30-May; 05-Jun; 13-Jun; 14-Jun; 19-Jun; 27-Jun; 01-Jul; 10-Jul; 22-Jul; 01-Aug; 09-Aug; 21-Aug; 03- Sep; 11-Sep; 21-Sep; 07-Oct; 14-Oct; 22-Oct	30-Apr; 07-May; 13-May; 21-May; 31-May; 07-Jun; 14-Jun; 20-Jun; 28-Jun; 08-Jul; 16-Jul; 23-Jul; 29-Jul; 02-Aug; 09-Aug; 23-Aug; 29-Aug; 05-Sep; 13-Sep; 26-Sep; 15-Oct

TABLE 2 | Species frequency (number of samples where the species was present N and expressed as the percentage of the total number of samples of samples where native coccinellid species occurred (N_{total}) and species abundance (average number of individuals per 100 sweeps ± SE) in samples where the native species occurred (N_{total}).

	Outskirts $N_{total} = 324$ (448)		Center $N_{total} = 180$ (454)	
	Frequency N (%)	**Abundance Mean ± SE**	**Frequency N (%)**	**Abundance Mean ± SE**
Adalia bipunctata	13 (4.0%)	1.1 ± 0.10	14 (7.8%)	1.1 ± 0.12
Adalia decempunctata	186 (57.4%)	4.0 ± 0.30	113 (62.8%)	3.6 ± 0.39
Anatis ocellata	5 (1.5%)	1.1 ± 0.19	2 (1.1%)	1.0 ± 0.00
Aphidecta obliterata	63 (19.4%)	2.8 ± 0.45	18 (10.0%)	1.2 ± 0.16
Calvia decemguttata	48 (14.8%)	1.8 ± 0.18	17 (9.4%)	1.2 ± 0.10
Calvia quatuordecimguttata	67 (20.7%)	1.7 ± 0.12	13 (7.2%)	1.8 ± 0.31
Ceratomegilla undecimnotata	1 (0.3%)	1.0	0	0
Chilocorus bipustulatus	4 (1.2%)	1.4 ± 0.22	5 (2.8%)	1.0 ± 0.03
Coccinella septempunctata	140 (43.2%)	3.0 ± 0.39	39 (21.7%)	4.4 ± 1.40
Exochomus quadripustulatus	27 (8.3%)	1.7 ± 0.20	1 (0.6%)	1.0
Halyzia sedecimguttata	6 (1.9%)	1.7 ± 0.72	1 (0.6%)	1.0
Harmonia quadripunctata	11 (3.4%)	1.4 ± 0.19	14 (7.8%)	1.3 ± 0.23
Hippodamia variegata	20 (6.2%)	1.5 ± 0.29	0	0
Oenopia conglobata	38 (11.7%)	1.4 ± 0.11	26 (14.4%)	1.9 ± 0.30
Propylea quatuordecimpunctata	67 (20.7%)	1.5 ± 0.11	31 (17.2%)	1.2 ± 0.10
Rhyzobius litura	35 (10.8%)	4.7 ± 1.63	0	0
Scymnus sp.	14 (4.3%)	1.6 ± 0.28	0	0
Subcoccinella vigintiquatuorpunctata	8 (2.5%)	1.0 ± 0.16	0	0
Thea vigintiduopunctata	3 (0.9%)	1.3 ± 0.47	0	0
Tytthaspis sedecimpunctata	0	0	1 (0.6%)	1.0
Vibidia duodecimguttata	1 (0.3%)	1.8	0	0

In parentheses: The total number of samples collected, including samples where no native coccinellids were present.

All species (regardless of their trophic specialization) were used to calculate the Shannon index describing species composition: Shannon index of diversity, H_0, calculated as $H_0 = \Sigma(pi * \ln(pi))$, where pi is the proportion of the total number of captured individuals of species i.

To compare the frequency and abundance of species, the results were transformed into *z*-scores. The values of characteristics (i) and (ii) were standardized by transformation to *z*-scores, which is the distance of a count from the mean value in standard deviation units. *Z*-scores were calculated using the formula $z = (x - m)/s$, where x is the arithmetic mean of the values of characteristics (i) or (ii) on each sampling occasion, m are the values of characteristics (i) or (ii) on each sampling occasion and s is the standard deviation of characteristics (i) or (ii) on each sampling occasion.

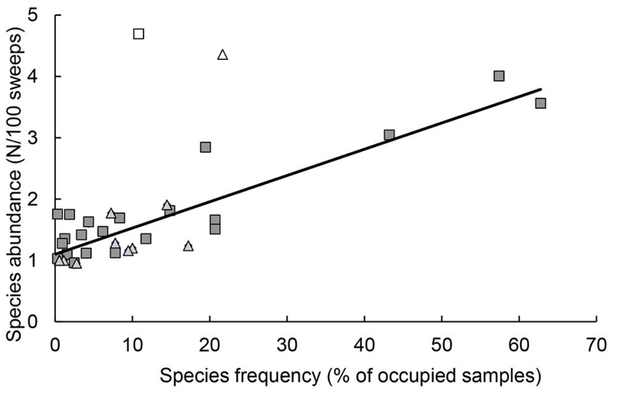

FIGURE 2 | Relationship between the two characteristics of the presence of coccinellid species, species abundance (average number of individuals per 100 sweeps) and species frequency (the percentage of samples in which the species was found). Each symbol represents one species of coccinellid, in the species composition in the outskirts (■) and center (▲). Hollow symbols: outliers not included in the calculation of regression (☐), *Rhyzobius litura* (Δ), *Coccinella septempunctata*. Regression: $y = 0.0155x + 1.1192$, $R^2 = 0.7944$, $n = 32$, $P < 0.001$.

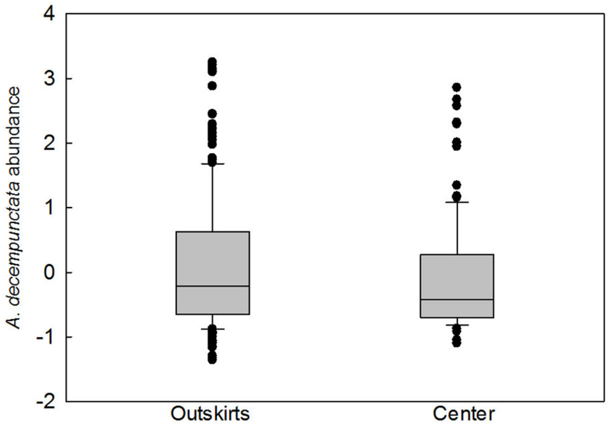

FIGURE 3 | The difference in abundance (number of individuals per 100 sweeps transformed as z scores) of *Adalia decempunctata* in the outskirts and in the center ($P_{MW} = 0.050$, U = 11931.000). The graph plots the median and the 10th, 25th, 75th, and 90th percentiles as vertical boxes with error bars and outliers (dots).

The differences between means were tested by *t*-test or, when the normality test failed, by the Mann-Whitney (MW) test. To test for the difference in species occurrence in the outskirts vs. centers, a *t*-test was used for paired values, where the response variable was characteristic (i) (in angular transformation). The relationship between variables was fitted by a linear regression of $y = ax + b$. All calculations were performed using SigmaStat 3.5 software (Systat Software Inc, 2006).

RESULTS

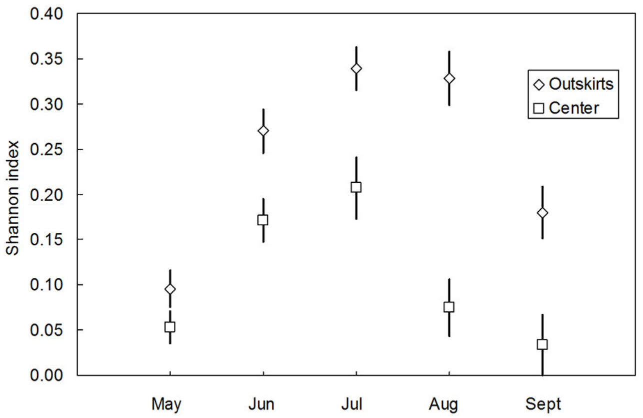

FIGURE 4 | Species diversity (mean Shannon index $H_0 \pm$ SE) over the course of the vegetative season (May–September) of coccinellid communities in the outskirts and center (pooled data from 2016–2019). The differences throughout the vegetative season: May (Nsmall = 42, Nbig = 54, U = 1302.500, $P_{MW} = 0.113$, N.S.), June (Nsmall = 62, Nbig = 80, U = 3236.500, $P_{MW} = 0.006$), July (Nsmall = 46, Nbig = 80, U = 2425.000, $P_{MW} = 0.003$), August (Nsmall = 21, Nbig = 64, U = 1082.000, $P_{MW} < 0.001$), and September (Nsmall = 9, Nbig = 46, U = 292.500, $P_{MW} = 0.034$).

The frequency of samples containing native species was higher in outskirts (324 samples out of a total of 448 samples collected, i.e., 72%) than in the centers (180 samples out of a total of 454 samples collected, i.e., 40%) (**Table 2**). A total of 2,761 adults, 1,909 in the outskirts, and 807 in the center, of 21 species of native coccinellids were found in the outskirts and center. Thirteen species were found in both areas, seven species were found only in the outskirts and were missing in the center, and one species was found only in the center and was missing in the outskirts (**Table 2**). Of the species found in both areas, most were more frequent in samples from the outskirts than in samples from the center: e.g., *Coccinella septempunctata* L. (found in 43% of samples from the outskirts and in 22% of samples from the center), followed by fewer species of *Aphidecta obliterata* (L.) (19 and 10%, respectively), *Calvia decemguttata* (L.) (15 and 9%), *C. quatuordecimguttata* (L.) (21 and 7%), and *Propylea quatuordecimpunctata* (L.) (21 and 17%). In contrast, *Adalia decempunctata* (L.) was more frequent in samples from the center than from the outskirts (63 and 57%), similar to *Adalia bipunctata* (L.) (8 and 4%), *Harmonia quadripunctata* (Pontoppidan) (8 and 3%), and *Oenopia conglobata* (L.) (14 and 12%). Some species were found at low frequencies in the outskirts and center: *Anatis ocellata* (L.) (2 and 1%), *Chilocorus bipustulatus* (L.) (1 and 3%), and *Halyzia sedecimguttata* (L.) (2 and 1%). Of the species missing from the center, *Rhyzobius litura* (Fabr.) (11%) and *Hippodamia variegata* (Goeze) (6%) were moderately abundant in the outskirts. In species present in both areas, abundance (characteristic ii) was greater in the outskirts in eight species (*Adalia decempunctata*, *Anatis ocellata*, *Aphidecta obliterata*, *Calvia decemguttata*, *Chilocorus bipustulatus*, *Exochomus quadripustulatus* (L.), *Halyzia sedecimguttata*, *Harmonia quadripunctata*), equal in both areas in one species (*Adalia bipunctata*), and greater in the center in three species (*Calvia quatuordecimguttata*, *Coccinella septempunctata*, *Oenopia conglobata*). A significant difference in species presence between outskirts and center was found only in *A. decempunctata*: the frequency (characteristic i) in the outskirts and center populations did not differ significantly (results not shown), and only abundance (characteristic ii) was significantly higher in the outskirts than in center populations (**Figure 3**).

Communities of coccinellids from the outskirts were richer in species (containing 20 species) than communities from the center (13 species). The presence of species in the communities of both areas (characteristic i) differed significantly [t(paired values) = 2,513, $n = 21$, $P < 0.05$]. The species diversity of communities in the outskirts was higher than that in the center. Expressed as the Shannon index (H_0), the differences were significant in individual months except during May (**Figure 4**) and over the entire vegetative season (Nsmall = 180, Nbig = 324, U = 41907.000, $P_{MW} < 0.001$). In the center, *A. decempunctata* and *C. septempunctata* significantly dominated the other species (**Table 1**).

DISCUSSION

The urban area (center), compared to outskirts, appears at first glance as an environment hostile to coccinellids. This is especially true in agglomerations where most of the area of the center is impervious, covered with buildings or paved roads, and without urban green spaces. However, some cities, including Prague, are less hostile to coccinellids because of continuous green spaces (parks, gardens) and tree lines planted on the impervious surfaces of the streets. Coccinellids live in these small areas of urban green spaces, often in high concentrations (Honek et al., 2017; Rocha et al., 2018). A factor that positively affects the aggregation of coccinellids in the city is the restriction of urban green areas to small areas, whereas populations outside the city would be evenly scattered over a large area instead of being concentrated. The urban environment *per se* may favorably affect the living conditions of coccinellids. One positive effect may be the temperate microclimate of the center and the smaller difference between day and night temperatures in the urban area than in the outskirts (Bonan, 2002). Additionally, the aphid abundance in urban tree stands may be higher than that in stands growing in the country, probably due to stress, which makes host trees less resistant to aphids (Fluckiger and Braun, 1999; Mackos-Iwaszko et al., 2015).

Despite the potential positive effects of urbanization, in this study, the coccinellid communities of the center were less species-rich and less abundant than the communities of the outskirts.

The mechanism of the effects of urbanization on the abundance of native coccinellids can only be speculated. If there is a regular dispersion of native species from the outskirts to the center, then the absence or low frequency of some species may be caused by spatial isolation of *Tilia* stands in the center and the distance from source populations of native coccinellid species in the outskirts. Dispersions that manifested in the center of Prague were observed in *C. septempunctata*. In the 1980s, they occurred regularly in late July when the adults flew to hibernation sites after the end of the period of mass reproduction in crop fields (Honek, 1989). Significant flight activity was also found in *A. bipunctata*, *A. decempunctata*, and *H. quadripunctata* (Honek, 1977), but its causes, timing, duration, and distance were not established.

The abundance of native coccinellids can be also negatively affected by the invasive *Harmonia axyridis* (Roy et al., 2012; Brown et al., 2015; Kenis et al., 2017; Masetti et al., 2018; Zaviezo et al., 2019). The extent of this negative effect depends on the species of native coccinellid (Honek et al., 2019a; Sloggett, 2021) and on the abundance of *H. axyridis* (Honek et al., 2018a, 2019b). If the occurrence of *H. axyridis* and its effects on native species differ, this invasive species could significantly affect differences in the presence and abundance of native coccinellids. The available data do not allow verification of this effect, as long-term records of the frequency of native coccinellids and *H. axyridis* are not yet available (Honek et al., 2020).

It can be concluded that the urban environment negatively affected coccinellid communities. The negative effect manifested even though the Prague agglomeration is relatively small (∼1 million inhabitants) and, in the center, impervious surfaces take only approximately three quarters of the surface area. To evaluate the impact of urbanization on coccinellid communities in general, it is necessary to gather more data on cities that have different sizes and different proportions of built-up areas than those in the center of Prague.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JS and AH designed the experiments, collected data, analyzed the data, and performed the statistical analyses. ZM coordinate all work. All authors wrote and edited the manuscript.

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ORIGINAL RESEARCH
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The Rare Five-Spot Ladybird *Coccinella quinquepunctata* (Coleoptera: Coccinellidae) Surviving in an Unstable Habitat

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Coccinella quinquepunctata (the five-spot ladybird), was considered extinct in the UK until 1987. Since this time the species is abundant, however, only in very specific habitat in Wales and Scotland. As a result, it is classified as (RDB3) Rare, mainly as a result of its preferred habitat; exposed riverine sediment. This habitat is in a constant state of alteration by natural and anthropogenic means with the quality of the habitat being degraded to the point that specialised invertebrate species, such as *C. quinquepunctata*, are at risk. In recent years, the rapid spread of the invasive alien *Harmonia axyridis* (harlequin ladybird) has been linked to a decline in native coccinellid numbers. There is concern that the narrow habitat requirements of *C. quinquepunctata*, together with the continuing spread of *H. axyridis*, will result in a decline in the abundance of *C. quinquepunctata*. Two habitat types (exposed riverine sediment and grassland adjacent to the ERS) along 12 Welsh rivers were surveyed for *C. quinquepunctata*, *H. axyridis,* and other coccinellids. When an individual coccinellid was recorded, so too was its elevation from the substrate. Plant species that *C. quinquepunctata* were observed on and vegetation density on the shingle were assessed in broad categories. Of all recorded coccinellids, 76% were *C. quinquepunctata* while 7% were *H. axyridis*. A third of the sites had no records of *H. axyridis,* while *C. quinquepunctata* was recorded at all sites. A significantly greater number of *C. quinquepunctata* were observed within 0.5 m of the exposed riverine sediment rather than higher up on the vegetation. Presence of the invasive plant Himalayan balsam (*Impatiens glandulifera*) may have a negative effect on *C. quinquepunctata,* as it directly affects the vegetation growth on expose riverine sediment. These findings indicate that intraguild predation is unlikely to occur given the low abundance of *H. axyridis* in *C. quinquepunctata* habitat. However, the unstable nature of exposed riverine sediment, and a combination of threats from invasive alien species indicates that this species is still at risk of sudden decline and requires further monitoring and conservation efforts.

Keywords: 5-spot ladybird, *Coccinella quinquepunctata*, exposed riverine sediment, *Harmonia axyridis*, invasive alien species, unstable river shingle

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INTRODUCTION

Coccinella quinquepunctata Linnaeus (five-spot ladybird) (Coleoptera: Coccinellidae) is a small conspicuous coccinellid, typically about 5 mm in length and red with black spots. This species is not found in Ireland, whilst in the UK, *C. quinquepunctata* is only found in limited areas within restricted habitat of unstable river shingle, also known as exposed riverine sediment (ERS), in Wales and Scotland (Roy et al., 2011). Due to very few records since 1913, *C. quinquepunctata* was considered extinct in the UK until 1987 (Majerus and Fowles, 1988). As a result of the restricted distribution of *C. quinquepunctata* in the UK, this species falls under the Red Data Book Category 3 (RDB3) Rare. The RDB3 classification is for taxa that are not yet endangered or vulnerable but are at risk due to restrictions in their habitat or geographical area (Hyman, 1992). Upon the rediscovery of *C. quinquepunctata* in the UK, more information became available regarding vegetation that this species was associated with on the shingle banks. It was also noted that the species was more likely to be observed on low vegetation, not more than 30–45 cm in height (Majerus and Fowles, 1988). In the late 1980s, surveys reported the species to be well-established in west Wales on both the River Ystwyth and Rheidol as well as in south east Wales on River Towy, with reports of up to 50 individuals recorded at some sites (Majerus and Fowles, 1988). *Coccinella quinquepunctata* was easily found on thistle or dock growing on river shingle along the River Towy and River Severn in 2002 and 2003 (Bates and Sadler, 2004). In Scotland, there were previous records of *C. quinquepunctata* in the early 1900s (Majerus and Fowles, 1988) and upon the rediscovery in Wales, surveys were subsequently undertaken at previously recorded sites on the River Spey in Scotland. Since then, other sites of suitable habitat have been identified along the River Dee and surveys carried out resulting in further observations of this species in Scotland (Littlewood, 2015).

Climatic conditions in the UK are considered suboptimal for some coccinellids, resulting in the UK being the edge of the acceptable range for several coccinellid species (Brown and Roy, 2015). In central Europe, *C. quinquepunctata* is not limited to ERS and is found in more generalist habitats such as trees, wild herbaceous vegetation and cereal fields (Honěk et al., 2014; Majerus et al., 2016). Exposed riverine sediment is in a constant state of alteration due to the nature of the river systems and water levels rising and falling regularly (O'Callaghan et al., 2013). Water levels not only rise in terms of depth but water also moves inwards across the shingle to the extent of the terrestrial habitat during high/maximal flow periods. The water level can rise and fall in this way quite quickly (several metres in 30 min), depending on the underlying geology of the upstream river catchment (Baker et al., 2004). As a result, the invertebrate community in these habitats is well-adapted to the unpredictability of these shingle banks (Sadler et al., 2004). Bates and Sadler (2004) have described *C. quinquepunctata* as having an ERS fidelity grade of 1, like many invertebrates inhabiting ERS. This essentially means that in the UK *C. quinquepunctata* is dependent on unstable river shingle for at least one stage of its life cycle and is not found in other habitat unless it happens to resemble ERS in some way, for example lakes that have wave action resulting in a sediment similar to ERS (Bates and Sadler, 2004; Sadler et al., 2004). However, due to anthropogenic disturbances such as gravel extraction, livestock access, channel modification and the establishment of invasive alien species (IAS), the quality of ERS habitat is being degraded to the point that specialised invertebrate species are at risk (Hyman, 1992; Hewitt et al., 2010). *Impatiens glandulifera* (Himalayan balsam) is an invasive alien herbaceous plant that is one of the tallest in the UK reaching 2.5 m in height (Beerling and Perrins, 1993). This IAS outcompetes native plants by blocking light for low-growing plant species (Pyšek and Prach, 1995; Tang et al., 2014). Additionally, *I. glandulifera* alters the microbial soil community, making it difficult for native plants to take root (Pattison et al., 2016). Furthermore, the annual nature of *I. glandulifera* and its root structure, work together to de-stabilise the river bank leaving it more susceptible to erosion during flooding (Pyšek and Prach, 1995; Welsh Institute for Sustainable Environments Network, 2014).

Another IAS that may have a negative impact on *C. quinquepunctata* is *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae). *Harmonia axyridis* is now globally established either as a result of its use as biological control of pest aphids and coccids or accidental introduction. *Harmonia axyridis* is now a threat to native coccinellids and other non-target species (Harmon et al., 2007; Adriaens et al., 2008; Brown et al., 2011a; Losey et al., 2012; Grez et al., 2016; Honěk et al., 2016; Sloggett, 2017), however it is yet to be determined if this IAS is a threat to *C. quinquepunctata*. Several other coccinellids have also been used as biological control agents, with some spreading to non-target habitats as *H. axyridis* has, resulting in adverse effects on native species (Evans, 2000; Koch and Galvan, 2008; Roy et al., 2016) and thereby becoming invasive. The decline of *Adalia bipunctata* Linnaeus (Coleoptera: Coccinellidae) over a broad geographic range in North America became apparent after the invasion of *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) and *H. axyridis* (Harmon et al., 2007). Intraguild predation (IGP) occurs when the competition between two predators of the same prey results in one of those predators preying on the other (Polis et al., 1989). The two main factors affecting the direction of IGP are body size and trophic specialisation, where the biggest and less specialised species are more likely to act as the predator and the smaller and more specialised species become the prey (Polis et al., 1989). If there is a lack of prey for *H. axyridis* it may turn to intraguild predation and prey upon the eggs and larvae of other coccinellid species (Brown et al., 2011a; Roy et al., 2011). Intraguild predation has frequently been observed in coccinellid species, especially when *H. axyridis* is present (Pell et al., 2008; Lucas, 2012). In laboratory trials with 11 other coccinellid species, *H. axyridis* was the dominant predator in the majority of intraguild interactions, including when the IAS was paired with *C. quinquepunctata* (Ware and Majerus, 2008). In comparison to *H. axyridis*, *C. quinquepunctata* larva are smaller and do not possess defensive spines.

Regardless of other threats affecting native coccinellids, *H. axyridis* is a factor in how coccinellid communities have changed over recent years (Brown and Roy, 2018; Honěk et al., 2019).

This is concerning, as a diverse native coccinellid assemblage delivers invaluable services to their habitat by controlling aphids, coccids and other insect herbivores (Sloggett et al., 2008; Grez et al., 2014). It is likely that *C. quinquepunctata* carries out such a role for the plant community that survives on ERS. If *H. axyridis* were to become abundant or even dominant in this habitat, it is possible that, together with other invasive threats, this habitat would become irreparably damaged thereby negatively effecting *C. quinquepunctata* and the wider community of specialised invertebrates (Sadler et al., 2004). Although described as semi-arboreal, *H. axyridis* has been recorded in a very wide range of habitats in the UK: urban areas and gardens, grassland, arable land and deciduous, and coniferous woodland (Brown et al., 2011b). Additionally, it is not yet clear if *H. axyridis* can adequately compensate for the role that native coccinellids play in biological control should local extinctions occur (Roy et al., 2012). Research focusing on the impact of an IAS tends to concentrate more on native species that were once abundant and have noticeably declined since the establishment of an IAS, such as *Coccinella novemnotata* Herbst (Coleoptera: Coccinellidae) in North America (Losey et al., 2012; Tumminello et al., 2015) and *A. bipunctata* in the UK (Brown and Roy, 2018). Research that investigates specialist or rare coccinellid species that may be at risk of local/national extinction as a result of IAS is uncommon. Although classified as rare and low in abundance, *C. quinquepunctata* is stable in the UK (Brown and Roy, 2015; Roy et al., 2018), however, this species may be particularly susceptible to negative impacts from *H. axyridis* through competition for prey and IGP (Roy et al., 2016), if they co-occur. Aside from the short studies above, few details are known about the one of UK's rarest and most specialist coccinellid species. The aim of this study was to discover more about the ecology of *C. quinquepunctata* and if this nationally rare species may be at risk from *H. axyridis.* It was expected that *C. quinquepunctata* would be recorded in low numbers and that *H. axyridis* would be present in relatively high numbers.

MATERIALS AND METHODS

Field Sites

Twelve sites along the Rivers Severn, Towy, Usk, and Wye in Wales were surveyed (**Table 1**) in 2017 in mid-June, mid-August and late-September. All sites were surveyed at least twice but poor weather conditions resulted in just eight of the sites being surveyed for a third time. Ordnance Survey location was recorded using Garmin GPSmap 60CSx (https://buy.garmin.com/en-GB/GB/p/310). In order to standardise data collection, surveys took place between 10:00 and 16:00 when weather conditions were favourable. As such, data collection was carried out when the temperature was above 14°C, weather conditions were dry and wind speeds were below 5 on the Beaufort scale (Met Office, 2016). Humidity and ambient temperature were recorded using an EasyLog EL-21CFR-2-LCD (https://www.lascarelectronics.com/easylog-el-21cfr-2-lcd). Any gaps in the temperature/humidity data were completed with data sourced from the Met Office.

TABLE 1 | Location of 12 sites surveyed in 2017.

Location	Codes	Grid Refs.	River
Hay-on-Wye	HW	SO22964 42815	Wye
Glasbury	GL	SO17930 39176	
Llandinam	LL01	SO02206 89053	Severn
	LL02	SO02727 89387	
	LL03	SO02562 89828	
Llandovery	LLGC	SN75424 33439	Towy
	LLCW	SN74434 32124	
The Bryn	BR01	SO33073 09419	Usk
	BR02	SO33329 09571	
	BR03	SO34237 08932	
Abergavenny	AB01	SO29276 13866	Usk
	AB02	SO29761 13667	

Survey Methods

Sweep-netting was used to survey for coccinellids in vegetation adjacent to the ERS (**Figure 1**). This method involves the use of a sweep net which is a white canvas bag (46 cm diameter circular aperture) attached to a metal ring on a long pole. One sweep was carried out for 1 m of distance walked. The net contents were checked every 5 m for coccinellids, which were recorded and the net subsequently emptied. Individuals were shaken from the net in the opposite direction to that of further recording to avoid double counting. This was carried out 20 times resulting in 100 m of grassland being surveyed at each site. Sweep-netting this size of area took ∼20 min. *Coccinella quinquepunctata* has adapted to move quickly if disturbed so instead of sweep netting, a direct search was employed to survey the ERS/shingle banks. Direct searching of ERS was carried out by one surveyor (RAF) for 1 h (30 min on one occasion when two surveyors were present). The search was carried out by moving from the water's edge to where the shingle bordered with grassland (became terrestrial in nature) and continued laterally over and back across the shingle (**Figure 1**). Where each survey started was dependent on time of day to ensure that the researcher's shadow did not disturb any *C. quinquepunctata* individuals prior to observation or impair detection of individuals. The area of ERS searched varied due to both changeable water levels and varying vegetation density throughout the season. The density of the vegetation on the shingle banks was assessed in broad categories based on percentage cover of the area surveyed: low (0–30%), medium (31–60%), or high (>60%). When *C. quinquepunctata* was recorded, the distance the individual was from the water's edge was recorded, as was the individual's elevation from the substrate. The vegetation/grassland adjacent to the ERS was relatively low (no more than 1 m high) and included grasses, wildflowers, thistle, bramble, etc. All coccinellids encountered during both sweep-netting and direct searches were recorded. Some coccinellid species were later grouped together to form the category "Other" as there were too few of each species to apply meaningful analysis to. These species were *Propylea quattuordecimpunctata* Linnaeus (Coleoptera: Coccinellidae), *Tytthaspis sedecimpunctata* Linnaeus (Coleoptera: Coccinellidae), *Psyllobora vigintiduopunctata*

FIGURE 1 | Illustration of a typical site with ERS/shingle bank bordered by grassland/pasture with the start point for direct search highlighted.

Linnaeus (Coleoptera: Coccinellidae), and *Subcoccinella vigintiquattuorpunctata* Linnaeus (Coleoptera: Coccinellidae).

Data Analysis

The analyses were carried out using R Studio (R Core Team, 2018). The following R packages were used for basic analyses and visualisation of data: dplyr (Wickham et al., 2019), ggfortify (Horikoshi and Tang, 2016; Tang et al., 2016), ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2018). Wilcoxon paired tests were used to compare the abundance of *C. quinquepunctata* and *H. axyridis* abundance on ERS and also in the grass habitat. The following R packages were used for regression analyses: fmsb (Nakazawa, 2018), lmtest (Zeileis and Hothorn, 2002), pscl (Zeileis et al., 2008), sandwich (Zeileis, 2004, 2006), lattice and MASS (Venables and Ripley, 2002).

Regression Analysis

Generalised linear models (GLMs) were utilised to investigate the effects on coccinellid abundance of habitat (ERS or grass), month (Visit–June, August, September), coccinellid diversity (Shannon diversity), and vegetation cover (Cover). Environmental variables (temperature, humidity) were included in the models. When applying a GLM to count data, the results can often be overdispersed. Overdispersion happens for various reasons with the most common being excess zeros in the data (Beckerman et al., 2017). In the case of these data, overdispersion was common and so alternative regression models were applied to the data and a subsequent model selection carried out to determine which was the best fit, if any. The regression models [poisson, negative binomial (NB), zero-inflated poisson (ZIP) model and zero-inflated negative binomial regression (ZINB) model] were applied to the data. The zero-inflated models treat the zeros differently, either as true or false zeros (Zuur et al., 2012). True zeros occur because the habitat is not favoured by the organisms in question, for example, if winters are too harsh. False zeros on the other hand are when an individual was present but not recorded due to survey design or observer error. It is recommended that if a count dataset consists of true and false zeros then zero-inflated regression models should be applied (Zuur et al., 2012). Zero-inflated models can run using a poisson or negative binomial distribution. A zero-inflated model is essentially two models run at the same time, the count model (models the count data) and the binary model (models the zeros). Both parts of the model are fitted simultaneously and are modelled in terms of the explanatory variables (Zuur et al., 2007).

Data from ERS were analysed separately to the grassland data due to differences in sampling method. In the majority of cases, either a zero-inflated negative binomial (ZINB) model or negative binomial regression (NB) model were the best fit for the data, and on occasion the null model was the better fit. Starting with all variables in the model, a step-wise process was used to determine which variables had an impact on the dependent variable. Any variables resulting in a $p < 0.2$ were removed from the model. All models were compared to the null model, and reduced models compared with the full model. The z-statistic is used in these regression models as the variance is known, unlike in Gaussian models where the variance is estimated resulting in a t-statistic (Zuur et al., 2009). There are several methods to determine which is the best model to choose (e.g., Akaike Information Criterion, Bayesian Information Criterion) where the model with the lowest value is considered the best (Zuur et al., 2009; Beaujean and Morgan, 2016). In this study, log Likelihood, Akaike Information Criterion (AIC), and weighted AIC were utilised, with the weighted AIC being the deciding factor as to which model was the best fit. Temperature and humidity were checked for collinearity with a variance inflation factor (VIF). Neither variables were of concern with a VIF of <1.2 each, and both were incorporated into the regression models.

Shannon diversity was calculated for shingle and grass habitat separately and only for native coccinellid species. Simpson's diversity was not carried out as this measure is not as sensitive to rare species or those recorded in low numbers (Magurran, 2004; Morris et al., 2014) and there are instances in this dataset where there are several species recorded in low numbers. Differences in diversity across site types and season were calculated using *t*-tests, while ANOVA was used to assess any differences in diversity within the vegetation structure, followed by a *post-hoc* Tukey, if any significances were apparent. Regression models were applied to determine if native coccinellid diversity had any effect on the abundance of *C. quinquepunctata* and *H. axyridis*.

RESULTS

In 2017, nine coccinellid species were recorded at 12 river sites in Wales with 687 individuals being recorded

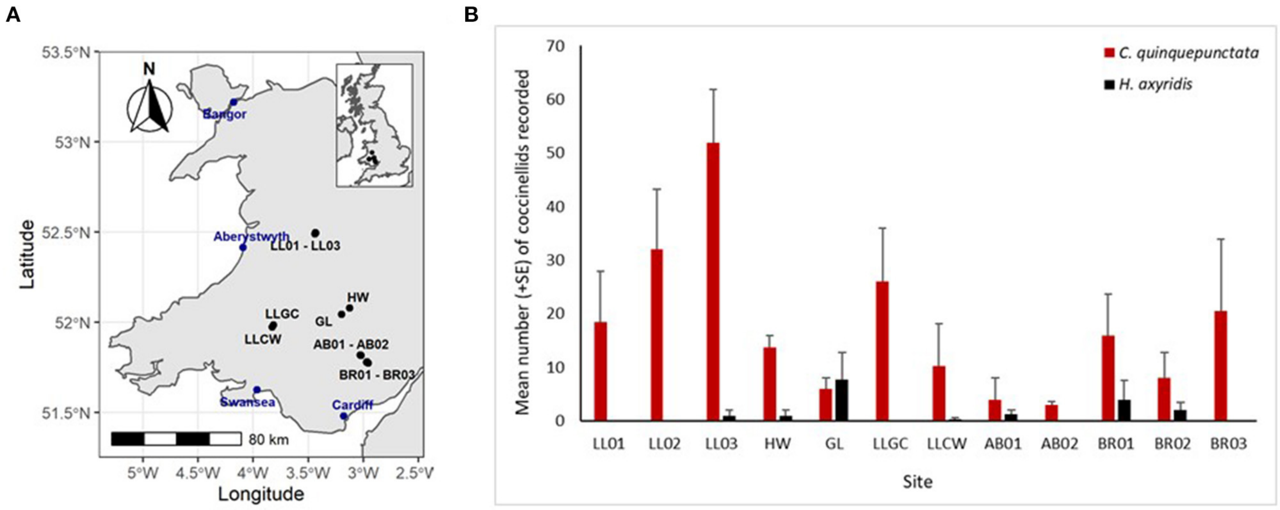

FIGURE 2 | (A,B) Map of survey sites along with mean number (+SE) per site visit of *Coccinella quinquepunctata* and *Harmonia axyridis* recorded at each site from both survey methods combined in 2017. Sites: AB01 and AB02, Abergavenny site 1 and 2; BR01, BR02, and BR03, Bryn sites 1, 2, and 3; GL, Glasbury; HW, Hay-on-Wye; LL01, LL02, and LL03, Llandinam sites 1, 2 and 3; LLCW, Cwmgwyn Farm; LLGC, Llandovery.

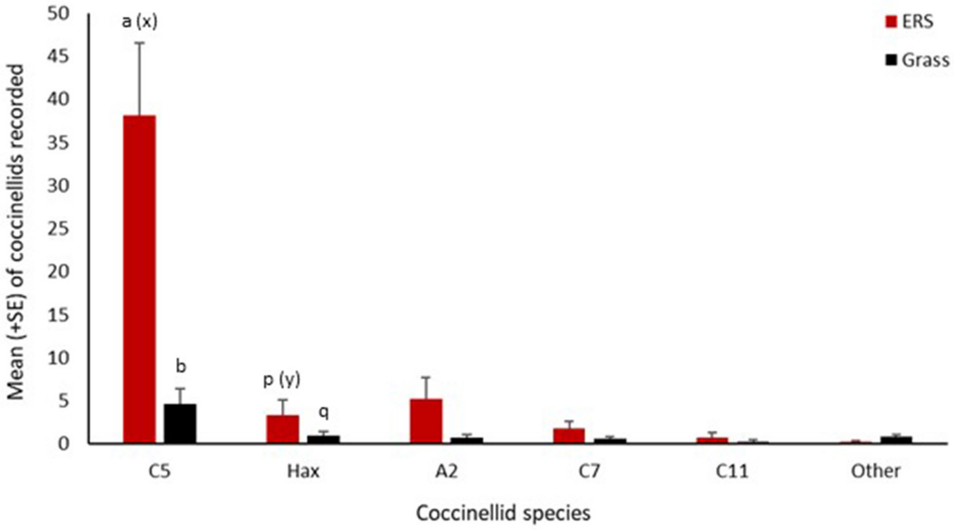

FIGURE 3 | Mean number (+SE) per site visit of coccinellids recorded on ERS and grassland in Wales in 2017. C5, *Coccinella* quinque*punctata*; Hax, *Harmonia axyridis;* A2, *Adalia bipunctata*; C7, *Coccinella septempunctata*; C11, *Coccinella undecimpunctata*; Other*, Propylea quattuordecimpunctata, Psyllobora vigintiduopunctata, Subcoccinella vigintiquattuorpunctata,* and *Tytthaspis sedecimpunctata.* Consecutive letters indicate where significant differences occur.

across both the shingle and grass habitat types. *Coccinella quinquepunctata* was present at all sites, while *H. axyridis* was only recorded at 7 of the 12 sites surveyed and was only more abundant than *C. quinquepunctata* at one site (**Figure 2**). A significantly greater number of *C. quinquepunctata* were recorded on the ERS habitat in comparison to the grass habitat ($z = 6.72$, $p < 0.0001$), however there was no such difference when comparing *H. axyridis* abundance at both habitat types (**Figure 3**).

Six species of coccinellid were observed through direct searching of the ERS habitat. In total, 592 coccinellids were observed by direct search with a large majority (77%) being *C. quinquepunctata* (**Figure 3**). The second most abundant coccinellid on ERS was *A. bipunctata* (10.5%) with *H. axyridis* (7%) being the third most abundant species. The abundance of *C. quinquepunctata* was significantly greater than that of *H. axyridis* on ERS habitat ($z = -4.32$, $p < 0.0001$) (**Figure 3**). Significantly more *C. quinquepunctata* were recorded in June ($z = 2.57$, $p = 0.01$) as opposed to in August and September. Coccinellid diversity had no effect on *C. quinquepunctata* numbers on shingle, nor did vegetation cover. There was no effect of season or vegetation cover on *H. axyridis* abundance on ERS. However, the reduced model revealed that *H. axyridis* abundance was higher when coccinellid diversity was higher ($z = 4.71$, $p < 0.0001$) on ERS.

Of the 95 coccinellids recorded in grassland adjacent to the ERS, the majority (58%) again were *C. quinquepunctata*. This habitat had a higher species richness than the ERS with nine species of coccinellid recorded. This difference was due in part to the presence of specialist coccinellids that are only found in grassland habitat (*P. vigintiduopunctata, S. vigintiquattuorpunctata*, and *T. sedecimpunctata*). There were significantly more *C. quinquepunctata* recorded in grassland than *H. axyridis* ($Z = -2.728$, $p = 0.02$) (**Figure 3**). In contrast to the ERS habitat, abundance of *C. quinquepunctata* was higher when coccinellid diversity was higher ($z = 2.99$, $p = 0.002$) in the grassland.

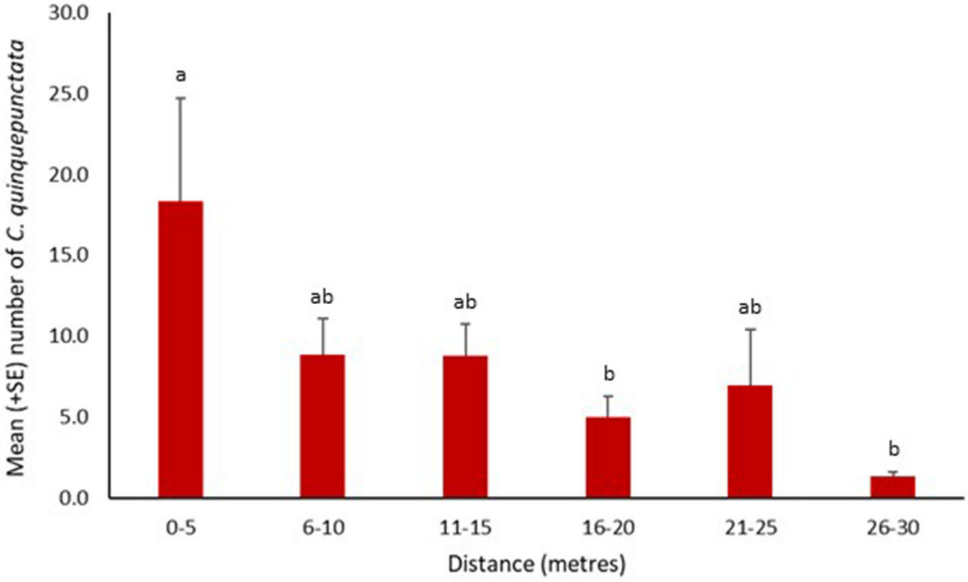

FIGURE 4 | Mean number (+SE) per site of *Coccinella quinquepunctata* and the distance (in m) from the water's edge they were recorded at in 2017. Consecutive letters indicate where significant differences occur.

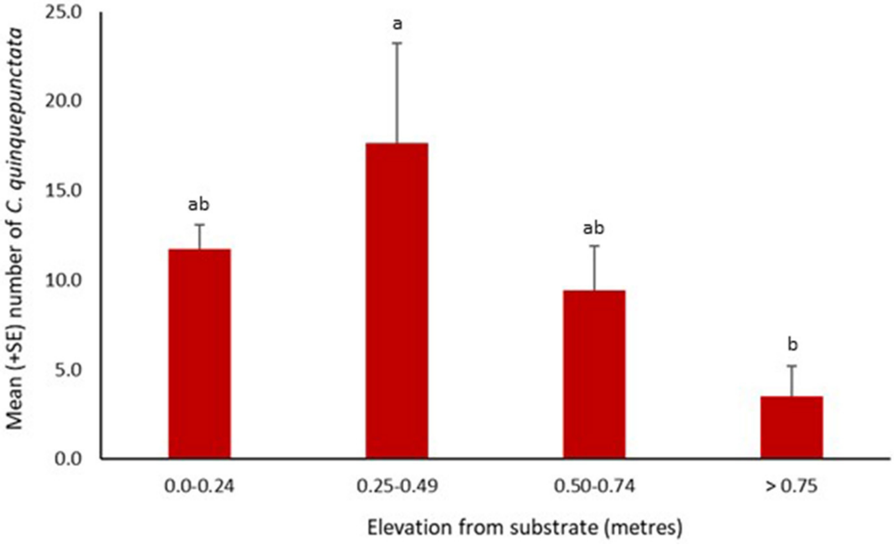

FIGURE 5 | Mean number (+SE) per site of *Coccinella quinquepunctata* and the distance (in m) from the substrate they were recorded at in 2017. Consecutive letters indicate where significant differences occur.

Coccinella quinquepunctata was found at a range of distances from the water's edge. Significantly fewer individuals were recorded further from the water's edge at 16–20 and 26–30 m ($z = -2.76$, $p = 0.006$ and $z = -3.51$, $p = 0.0004$, respectively). The numbers recorded at the other three distances were also lower but not significantly so (**Figure 4**). *Coccinella quinquepunctata* was observed at various heights from the shingle substrate but was found more frequently at lower elevations with significantly fewer individuals recorded above 75 cm from the ground ($z = -2.85$, $p = 0.004$) (**Figure 5**).

DISCUSSION

Coccinella quinquepunctata was recorded in higher numbers than expected during this study and more *C. quinquepunctata* were observed than *H. axyridis* on both the ERS habitat and grassland habitat. The generally rural nature of the habitat is likely to be less suitable for *H. axyridis* (Purse et al., 2014) and consequently a refuge for *C. quinquepunctata.* The low number of *H. axyridis* was surprising, however this species has a well-documented preference for urban habitats (Adriaens et al., 2008; Purse et al., 2014; Roy and Brown, 2015; Sloggett, 2017; Viglášová et al., 2017) and in this case, all sites surveyed were in rural areas or on the edge of small rural villages. Urban and other managed habitats are more suitable for *H. axyridis* by providing secure overwintering sites in buildings (Roy et al., 2011, 2016). Furthermore, the ERS is a unique habitat with sparse vegetation stands where aphid numbers perhaps may be too low to sustain a predator such as *H. axyridis*. Honěk et al. (2018) reported an increase in *H. axyridis* numbers when aphid numbers increased but also with an increase in the level of urbanisation. Considering the rural nature of the sites as well as lack of overwintering sites, it is interesting that *H. axyridis* was recorded at all. Native coccinellid species were present in both habitats and *A. bipunctata* was present on ERS in greater numbers than was *H. axyridis*, albeit not significantly so. The overall low number of other coccinellid species recorded further reiterates that ERS is not a particularly suitable habitat for most coccinellids. In the grass habitat the low number of *H. axyridis* mirrored that of the overall number of coccinellids. Despite the low number of coccinellids in grassland, there was a greater diversity of native coccinellids in this habitat than on the ERS. Even though this was most likely due to the presence of three grass-specialist coccinellid species, the difference was not significant. The time spent searching grass habitat was just less than half the time spent searching on ERS, which partially accounts for the lower numbers recorded. Additionally, it is not possible to directly compare the two sampling techniques. The number of *C. quinquepunctata*, however, was higher when coccinellid diversity was higher in the grass habitat only. This could be due to a very low number of *H. axyridis* recorded in the grass habitat. However, it is more likely that coccinellid diversity and abundance was higher where the habitat was less managed or disturbed thereby creating a more suitable habitat for coccinellids (Diepenbrock and Finke, 2013; Grez et al., 2014; Honěk et al., 2014). Further investigation into coccinellid diversity and the heterogeneity/disturbance of the habitat adjacent to ERS would reveal more about the interaction between *C. quinquepunctata* and other coccinellids as well as the native coccinellid community.

Prior research indicated that *H. axyridis* could represent a threat for this species (Ware and Majerus, 2008). Thus, it is likely that should IGP occur, it would have a negative effect on *C. quinquepunctata*. However, the results in this study indicate that habitat separation on ERS may limit the interactions of these two species thereby limiting the opportunities for IGP. In North America, threat in the form of IGP and competition for resources from *H. axyridis* exacerbated the situation with *C. novemnotata*, which is found in only a small number of states and where present are in greatly reduced numbers (Tumminello et al., 2015; Ducatti et al., 2017). In Europe, *C. quinquepunctata* was previously considered an abundant habitat generalist, however, a decline in abundance was evident prior to the arrival of *H. axyridis* (Honěk et al., 2016). It is thought that changes in land use together with the intensification of agricultural practises has impacted *C. quinquepunctata* in the Czech Republic (Honěk et al., 2016). The results here also indicate that *H. axyridis* is not currently impacting *C. quinquepunctata* negatively, due to very low abundance of the former in the preferred habitat of the latter.

Other threats are very likely to have a negative impact on *C. quinquepunctata* because of their impact on the ERS. The reason for the RDB3 (Rare) categorisation of this species is due to the habitat where it is found being at risk. There are several threats to this habitat; including invasive plant species, livestock access to shingle banks, gravel extraction and river modification (Fowles, 1988; Bates et al., 2007a; Hewitt et al., 2010). Multiple IAS and various anthropogenic activities together may culminate into drivers of change (Vitousek et al., 1997), and being clear on which factor happens to be the greatest threat will facilitate effective conservation plans for native species (Majerus et al., 2016). More than one invasive alien plant species was identified on or near the shingle habitat (e.g., Japanese knotweed, *Fallopia japonica*; monkey flower, *Erythranthe guttatus*), however, the species most likely to have the greatest negative and immediate impact is *I. glandulifera* (Himalayan balsam). Seven of the 12 sites surveyed here had established stands of *I. glandulifera* present. This species potentially impacts *C. quinquepunctata* in two ways. Firstly, *I. glandulifera* changes the microbial community of the soil which prevents native plant species from taking root (Pattison et al., 2016), thereby homogenising the ERS plant community. During surveys, neither aphids nor any coccinellid species were seen on *I. glandulifera* plants (pers. obs.). This is not surprising, given that Tanner et al. (2013) reported a reduction in coccinellid numbers on areas invaded by *I. glandulifera* in comparison to non-invaded areas. Considering the significantly reduced abundance of *C. quinquepunctata* in the grassland adjacent to the shingle, the potential and inevitable lack of native plant species as a result of the presence of *I. glandulifera,* potentially leading to insufficient prey for *C. quinquepunctata*, could see the species become locally extinct in areas where *I. glandulifera* is not adequately controlled. If this habitat becomes homogenised in terms of vegetation the specialised invertebrate community is likely to be negatively affected (Sadler et al., 2004). Additionally, *I. glandulifera* de-stabilises the shingle bank as it has shallow roots and the soil around it becomes more fragmented, so when the rivers are in flood, considerably more substrate than usual will be removed. The ERS is in a constant state of flux (Fowles, 1994), however, this increased threat is likely to have an adverse effect not just on *C. quinquepunctata* but also the many other invertebrates (many of which are also nationally rare) that inhabit the fragile habitat (Sadler et al., 2004). Livestock regularly have access to the ERS for water and also graze on the bank. This is likely to have a negative impact on *C. quinquepunctata* due to the additional disturbance of the ERS, given this species' reliance on this habitat type. Bates et al. (2007a) determined that trampling by livestock reduced the conservation value of the beetle assemblages on river shingle. However, a small number of sites in this research, that were grazed by sheep during the entire field season, yielded the highest number of observations of *C. quinquepunctata*. These sites, however, were also clear of *I. glandulifera* and Day (2015) reported that grazing can be used to help control *I. glandulifera* successfully. Nevertheless, this IAS can be readily removed by hand and uncontrolled livestock access is more likely to be negative rather than a positive influence for ERS. One of the sites in this study had gravel extracted from it just prior to the final survey. This process resulted in complete removal of the vegetation and a large layer of the shingle bank. This site was the closest site to an urban area and in addition to the gravel extraction, the vegetation was highly managed throughout the entire survey period. *Coccinella quinquepunctata* was present at the site but in lower numbers than elsewhere. If the vegetation had not been cut back so severely and so frequently, it is possible that a greater number of *C. quinquepunctata* would have been recorded. This degree of disturbance to the ERS habitat and adjacent grassland mainly as a result of gravel extraction is a serious concern for *C. quinquepunctata* and other ERS-dwelling invertebrates (Sadler et al., 2004; Bates et al., 2007b). This level of disturbance is especially concerning, considering that after river system modification took place, it was reported that all trace of ERS had disappeared from the midlands and south east of England (O'Callaghan et al., 2013).

In Europe, *C. quinquepunctata* is in decline, however, in the UK, though it has a very restricted range the species is reported as nationally stable (Roy et al., 2018) and the numbers recorded during this research support that assessment. However, several coccinellid species have declined in recent years (Roy et al., 2018) and it is unlikely that there are other factors driving trends alongside the co-occurrence with *H. axyridis*. There are numerous threats to ecological communities alongside biological invasions including climate and land-use change. It is clear that the drivers interact and lead to biodiversity change (Harvey, 2015) with different components of each community assemblage reacting differently to these multiple threats (Stewart et al., 2015).

FURTHER WORK

Continued monitoring of *C. quinquepunctata* (both in Wales and Scotland) is necessary to detect any future changes in the population. In the event of a decline in numbers, the continued monitoring of *H. axyridis* would further inform researchers if this IAS has started to have an effect on *C. quinquepunctata* or if a different threat may be having a negative impact. Monitoring will also help determine a more complete distribution in the UK. There does seem to be an edge of range effect in the UK. As a result of its exposure the ERS heats up quickly (Bates et al., 2009) which may be one factor as to why this habitat is preferred by *C. quinquepunctata*. This would be interesting to explore further in comparison to other habitat preferences for this species throughout Europe. Given that ERS is an important riparian habitat, additional studies into how the specialised invertebrate community contribute to ecosystem function would help bridge the gap between aquatic and terrestrial ecology in the UK. Finally, considering the specialist habitat preference of *C. quinquepunctata,* and numerous other rare invertebrate species, it would be prudent to designate increased habitat protection status on ERS in order to control livestock access, prevent gravel abstraction, river channel modification, and initiate restoration or enhancement of the habitat where it has been damaged or removed.

CONCLUSION

It is evident that *C. quinquepunctata* is thriving in Wales and is relatively unaffected by *H. axyridis* through IGP or resource competition. The RDB3 Rare categorisation is justified for *C. quinquepunctata* considering the multiple threats effecting ERS. However, if this unique habitat continues to be degraded, *C. quinquepunctata* is likely to decline, possibly to the point of extinction in the UK.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

RF, HR, and PB contributed to conception and design of the study. RF carried out field work, performed statistical analysis, and wrote the first draft of the manuscript. All authors contributed to revision of the manuscript and read and approved the submitted version.

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Double Infections of the Invasive Ladybird *Harmonia axyridis*

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The dramatic spread of invasive alien species over the past century is considered to be an important threat to ecosystems worldwide. The harlequin ladybird, *Harmonia axyridis*, from eastern Asia, is considered to be one of the most invasive species. Originally introduced across the world as a biological control agent against crop pests owing to its voracious appetite and hardiness, those same qualities have made *H. axyridis* an invader that is difficult to eradicate. *Harmonia axyridis* has proven resilient against a variety of pathogens that have negative effects on other coccinellids. However, little research has examined the effects of simultaneous infections of multiple natural enemies on *H. axyridis*. Here we present the available information on such double infections on *H. axyridis*, and discuss further research directions in this area.

Keywords: biocontrol, ecology, entomology, integrative pest management, invasion biology, parasitology

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INTRODUCTION

The introduction and dramatic spread of non-native species is seen as a significant environmental disturbance threatening ecosystems around the world (Mooney and Cleland, 2001; Simberloff and Gibbons, 2004). Human activity enables species to reach and establish themselves in regions outside their native range, known as biological invasions (Vitousek et al., 1996; Mack et al., 2000). Environmentally problematic species are increasing globally, even in areas that are traditionally seen as sources rather than targets of alien species, such as Europe (Hulme et al., 2009). Global trade and human movement exacerbate the rate of alien invaders establishing themselves in new environments, and this method of distribution is projected to accelerate in the future (Levine and D'Antonio, 2003; Pfliegler et al., 2018). Some invasive species have considerable negative environmental and socio-economic effects, leading to mounting efforts to mitigate the damage (Vilà et al., 2010).

One of the posited explanations for the success of invaders in new environments is the enemy release hypothesis (Keane and Crawley, 2002; Colautti et al., 2004; Liu and Stiling, 2006; Roy et al., 2011a). The enemy release hypothesis proposes that population growth of invasive species results from release from their natural enemies that did not co-evolve in the new geographic location with them. This is beneficial for the invasive species on two fronts: first, they are not regulated by specialists, and second, their direct competitors of similar ecological guilds do have specialist enemies. Over time, population dynamics will be affected by evolutionary processes and the amount of natural enemies of the invasive species can increase (Brändle et al., 2008; Schilthuizen et al., 2016; Haelewaters et al., 2017), but not necessarily in time before the ecosystem is harmed (Geschke, 2019).

The harlequin ladybird, *Harmonia axyridis* (Pallas) (Arthropoda: Coleoptera, Coccinellidae), is a ladybird native to eastern Asia. Due to its resilience and voracious appetite, it was deemed very useful as a biological control agent against aphids, coccids, and other pests, and has been widely used in horticulture (Koch, 2003). *Harmonia axyridis* was introduced to protect crops in North America and later as an augmentative biocontrol in Europe, but it has now spread at an explosive rate to every continent except Australia and Antarctica over the past 30 years (Brown et al., 2011; Roy et al., 2016; Camacho-Cervantes et al., 2017; Hiller and Haelewaters, 2019). The same qualities that make *H. axyridis* a successful biocontrol agent also make it an effective intraguild predator. Whereas *H. axyridis* in its native range reached an equilibrium with its co-evolved guild of predators, in its exotic range it is in direct competition with populations of native predators, as the enemy release hypothesis would predict (Pell et al., 2008).

Harmonia axyridis has a negative effect not only on native insects, such as causing local ladybird populations to decline (Mizell, 2007; Roy et al., 2012), but also on food production and human health (Koch, 2003; Pickering et al., 2004; Koch and Galvan, 2008). It has been described as one of the "worst" invasive alien species of Europe (Nentwig et al., 2018). The International Union for Conservation of Nature (IUCN) established a Ladybird Specialist Group to identify species that are threatened and to develop conservation management strategies to counteract factors leading to high extinction risk. New methods of *H. axyridis* control must be assessed for the IUCN and similar groups to succeed in their plans to protect threatened species. In assessing how invasive species like *H. axyridis* can be controlled, it is important to determine the natural enemies of *H. axyridis*, how they spread, and which role they may have in regulating invasive populations of *H. axyridis*.

The search for natural enemies of *H. axyridis* has identified a variety of pathogens, parasites, and parasitoids affecting the ladybird, including bacteria, protozoans, fungi, nematodes, mites, wasps, and flies (Ceryngier et al., 2012, 2018; Haelewaters et al., 2017). Most of these natural enemies are generalists for coccinellid species or other insects. Part of *H. axyridis*' competitive success may be explained by its high resistance to such generalist enemies; *H. axyridis* has a relatively greater efficiency of its immune system, giving it robust and flexible defenses that surpass other competing ladybirds in the same region (Roy et al., 2008, 2011b; Vilcinskas et al., 2013; Gegner et al., 2018; Fincham et al., 2019). The immune system of *H. axyridis* is two-layered—it combines constitutive chemical defenses effective against a variety of bacteria as well as a wide range of antimicrobial peptides that are a result of multiple gene duplication events after speciation (Vilcinskas et al., 2013). Additionally, *H. axyridis* possesses strong alkaloid chemical defenses against predators and pathogens, which give it a foul smell and taste. These *H. axyridis*-specific alkaloids have been found to be more toxic compared to other coccinellids (Röhrich et al., 2011; Sloggett et al., 2011).

While local natural enemies of ladybirds have started using *H. axyridis* as a new host in places where it is invasive (Raak-van den Berg et al., 2014; Knapp et al., 2019), some invasive populations of *H. axyridis* may be only rarely infected (Dudek et al., 2017; Romero et al., 2020). As a result, these enemies of *H. axyridis* only have limited biocontrol potential for controlling invasive populations on their own, and some may actually have more adverse effects on locally native species than on *H. axyridis* (Riddick, 2010; Haelewaters et al., 2017; Ceryngier et al., 2018). However, with this many potential natural enemies affecting *H. axyridis* and the species being so widespread, there might be combinations of pathogens, parasites, or parasitoids that have an adverse effect on the invasive populations. Here, we inventorize previously described double infections of *H. axyridis* and their effects, where known (**Table 1**).

DOUBLE INFECTIONS

Natural enemies impact their hosts' fitness in a variety of ways, such as increasing mortality, decreasing fecundity, and increasing susceptibility to parasites, pathogens, predators, or chemical agents. Insects are susceptible to infection by many different enemies, and simultaneous infections are anticipated to be common in the wild. In attempting to control invasive species, the additive effects of multiple infections can be a way to increase the efficiency of integrated pest management (IPM) programmes (Jabbour et al., 2011; Zindel et al., 2011). However, interactions between infecting species are difficult to predict, and can have conflicting results (**Figure 1**). For example, use of two species of natural fungal pathogens to combat the leaf-cutter ant *Acromyrmex lundii* (Guérin-Méneville) (Arthropoda: Hymenoptera, Formicidae), an agricultural pest in the Neotropics, caused the two species of fungi to suppress each other rather than the pest (Folgarait et al., 2011). In a another example, when the invasive garden ant *Lasius neglectus* Van Loon, Boomsma & Andrasfalvy (Formicidae) was exposed to the mortal entomopathogen *Metarhizium brunneum* Petch (Ascomycota: Hypocreales, Calvicipitaceae) after infection by *Laboulbenia formicarum* Thaxt. (Ascomycota: Laboulbeniales, Laboulbeniaceae), the upregulated immunological and behavioral responses caused fewer fatalities from the secondary infection than either fungus on its own (Konrad et al., 2015). Such complex interactions therefore warrant exhaustive review and experimentation, both by surveys in the wild and mortality experiments in laboratory settings, especially for species with hardy defense systems, such as *H. axyridis.*

The endosymbiotic bacterium *Spiroplasma* (Tenericutes: Entomoplasmatales, Spiroplasmataceae) infects the gut of hemolymph of a large variety of insects, with effects ranging from mutualistic to parasitic (Hackett and Clark, 1989). In a subset of insects including ladybirds, *Spiroplasma* causes so-called male-killing by destroying eggs fertilized by Y-bearing sperm, causing extremely female-biased offspring (Zakharov et al., 1999; Harumoto and Lemaitre, 2018). In *H. axyridis*, *Spiroplasma* also reduces body size, embryo survival, and adult lifespan in infected females (Majerus, 2002). Infection by *Spiroplasma* only seems to occur in native populations of *H. axyridis*; thus far infections have not been reported in populations in areas where they are invasive (Goryacheva et al., 2017). A survey of *Spiroplasma* strains in *H. axyridis* from Japan and eastern Russia—both in its native range—revealed the majority of beetles that were infected by *Spiroplasma* to be infected by multiple strains (88%) (Goryacheva et al., 2018). However, the exact

TABLE 1 | Known simultaneous infections by multiple natural enemies on *Harmonia axyridis* and their effect on the host.

Species 1	Species 2	Field or lab	Locality	Effect of co-infection	Reference(s)
Spiroplasma strain 1	*Spiroplasma* strain 2	Field	Japan, Russia	N/A	Goryacheva et al., 2018
Hesperomyces virescens	*Parasitylenchus bifurcatus*	Field	Germany, Netherlands, Caucasus	N/A	Herz and Kleespies, 2012; Raak-van den Berg et al., 2014; Orlova-Bienkowskaja et al., 2018
Hesperomyces virescens	*Coccipolipus hippodamiae*	Field, Lab	Austria, United States	Increased mortality	Christian, 2002; Riddick, 2010
Hesperomyces virescens	*Beauvaria bassiana*	Lab	United Kingdom, United States	No increased mortality	Berry, 2017; Haelewaters et al., 2020
Hesperomyces virescens	*Metarhizium brunneum*	Lab	United States	No increased mortality	Haelewaters et al., 2020

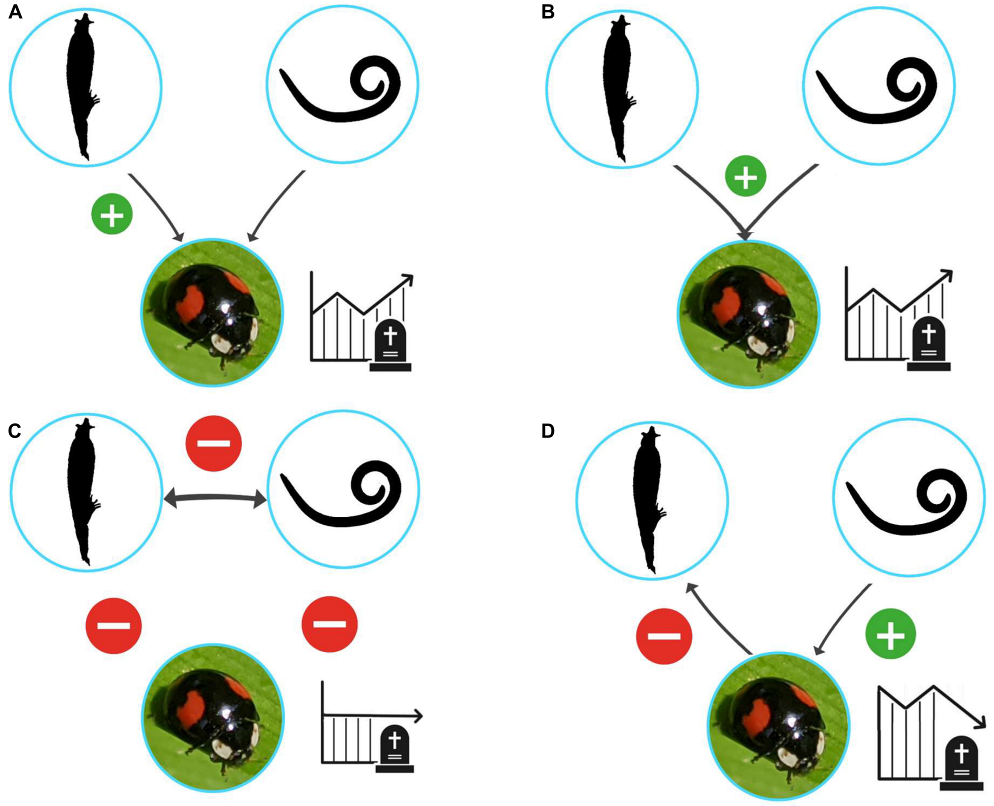

FIGURE 1 | Conceptual overview of potential effects of co-infection by two natural enemies on the fitness of a given host. **(A)** The co-infection causes no additional decrease in fitness. **(B)** There is an additive effect of the second enemy, causing a stronger decrease in fitness upon co-infection. **(C)** The infecting species neutralize each other, causing no increase or decrease in fitness. **(D)** One natural enemy causes a prophylactic effect in the host, preventing a secondary infection from taking hold, increasing the host's fitness. Photo: *Harmonia axyridis* (iNaturalist #53583172).

biological consequences of multiple infections as opposed to a single infection remain unclear.

The fungus *Hesperomyces virescens* Thaxt. (Ascomycota: Laboulbeniales, Laboulbeniaceae) is an obligate ectoparasite infecting adult ladybirds of over thirty species (Haelewaters et al., 2017). Recent integrative taxonomic analyses have revealed that *H. virescens* is in fact made up of many different species, each specifically adapted to its individual host (Haelewaters et al., 2018; Haelewaters and De Kesel, 2020; Crous et al., 2021). *H. virescens* completes its entire lifecycle on its living host; sexual spores divide mitotically to produce yellowish, multicellular, three-dimensional structures called thalli on the outside of any part of the host's body. These thalli penetrate the host's cuticle via a rhizoidal haustorium (De Kesel, 2011; Haelewaters et al., 2017). *Hesperomyces virescens* has been found to co-infect *H. axyridis* hosts alongside the nematode *Parasitylenchus bifurcatus* Poinar & Steenberg (Nematoda: Tylenchida, Allantonematidae) in Germany (Herz and Kleespies, 2012), The Netherlands (Raak-van den Berg et al., 2014), and the Caucasus (Orlova-Bienkowskaja et al., 2018). *Parasitylenchus bifurcatus* is an obligate endoparasite specific to *H. axyridis*; these nematodes live, mate, and proliferate within the host. The method of transmission from host to host is unknown (Poinar and Steenberg, 2012). It was speculated from observations in the field that simultaneous infection by

H. virescens and P. bifurcatus reduced survival rates of *H. axyridis* (Raak-van den Berg et al., 2014).

A different natural enemy to co-infect *H. axyridis* alongside *H. virescens* is the ectoparasitic mite *Coccipolipus hippodamiae* (McDaniel & Moril) (Arthropoda: Acarina, Podapolipidae). Simultaneous infections by these two natural enemies have thus far been observed in Austria (Christian, 2001, 2002) and the United States (Riddick, 2010). *Coccipolipus hippodamiae* infection causes decreased fecundity, decreased egg viability, and increased mortality in a variety of coccinellid species (Webberley et al., 2004), but the exact biological mechanisms are still unknown. Transmission from one host to the next occurs similarly to *H. virescens*: through bodily contact during mating or overwintering (Knell and Webberley, 2004). Mortality from *C. hippodamiae* infection is especially high in overwintering males (Webberley et al., 2006). In a simulated winter experiment, *H. axyridis* individuals co-infected by both mites and the fungus died earlier than those infected by the fungus only (Riddick, 2010).

Hesperomyces virescens has also been used to co-infect *H. axyridis* individuals in a laboratory experiment together with *Beauveria bassiana* (Bals.-Criv.) Vuill. (Ascomycota: Hypocreales, Cordycipitaceae) and *Metarhizium brunneum* Petch (Sordariomycetes: Hypocreales, Calvicipitaceae) (Berry, 2017; Haelewaters et al., 2020). *Beauveria bassiana* is among the best studied of the coccinellid pathogenic fungi. Germinating spores penetrate the host's integument, which is followed by mycelial proliferation inside the host's body. The fungus feeds on the host while it is alive. Infection is usually fatal for the host; after its death *B. bassiana* becomes saprophytic and produces conidia for further transmission (Ceryngier et al., 2012). While *B. bassiana* is considered a major mortality factor for many coccinellids, *H. axyridis* has been shown to lose fecundity under laboratory conditions, but mortality was not increased (Roy et al., 2008). Experiments co-infecting *H. axyridis* with *H. virescens* together with either *B. bassiana* or *M. brunneum* have no increased mortality in comparison to infection with only *H. virescens*. In contrast, the North American-native ladybird *Olla v-nigrum* (Mulsant) (Arthropoda: Coleoptera, Coccinellidae) was more susceptible to *B. bassiana* after infection by *H. virescens* (Haelewaters et al., 2020). This differential susceptibility between the native and invasive species is in line with the enemy release hypothesis, which, as detailed above, predicts that invasive species are so successful in part because they are less susceptible to the local natural enemies compared to native species.

DISCUSSION AND FUTURE DIRECTIONS

The above inventory of various pathogens and parasites co-infecting *H. axyridis* illustrate the fundamental gaps in our understanding of how these species interact on an ecological level, and that little research examines multiple groups of pathogens at once. Despite *H. axyridis* being a widely studied model organism for invasion biology (Roy and Wajnberg, 2008), little is known about its natural enemies. Additionally, the relatively minor effects on fitness by its natural enemies, even in simultaneous infections, have underlined the resilience of this invasive alien species. Still, that does not mean looking at co-infections is a fruitless endeavor, with some simultaneous infection combinations proving mortal in laboratory experiments. The surface of this area of research has barely been scratched, and further studies are warranted.

In nature, individual hosts are frequently infected by multiple pathogens, parasites, or parasitoids. However, the effects on survival rate and usability of a combination of natural enemies as biocontrol often remains unclear. One way to improve biological control of invasive and pest species is to exploit the potentially additive function of co-infections. Therefore, we need to understand (i) what biotic and abiotic factors determine the likelihood of a co-infection on a host, (ii) what the exact biological mechanisms are that cause double infections to have an additive negative effect on host survival or fecundity, and (iii) the ways in which these interactions affect the local ecology and non-target species.

This requires an approach that is neither a specifically pair-wise interaction between two species, nor a much larger-scale population-level analysis. This includes network approaches seeking to illuminate intra- and interspecific interactions between hosts and their multiple enemies (Roy and Lawson Handley, 2012). These interactions will likely also vary under different conditions and on different spatial and temporal scales. For example, fungal ectoparasites of ladybirds have been shown to be more prevalent in urban environments (urban heat island effect; Welch et al., 2001), and simultaneous infection can be extremely localized (Raak-van den Berg et al., 2014). To not just take a shot in the dark, it is therefore useful to identify which combinations of co-infecting species already exist in nature, and draw surveys and experiments from there. Molecular data should prove enlightening for unraveling the interactions between hosts and their various natural enemies—even more so for host–parasite interactions, as parasites are often cryptic, hidden, or inside the host, and therefore difficult to track by traditional surveying methods (Hesketh et al., 2010). Another potentially valuable avenue for collecting data on novel host–parasite interactions is found in citizen science; monitoring websites such as iNaturalist can provide reports of new occurrences of interactions between a host and its parasites, and in new localities (Haelewaters et al., 2019). Citizen science projects monitoring *H. axyridis* such as the Lost Ladybug Project in the United States and the United Kingdom Ladybird Survey, among others, also generate image collections that can be screened for parasites (Fothergill et al., 2010; Brown et al., 2018; Werenkraut et al., 2020). Similarly, digitized museum collections can be screened for ectoparasites using a stereoscope relatively easily (Báthori et al., 2017; Haelewaters et al., 2017).

Harmonia axyridis makes for a powerful model organism, both as an invasive alien species and for the study of simultaneous infections by natural enemies. While spreading around the world, to every continent except Australia and Antarctica, *H. axyridis* may have brought its natural enemies with it and it has interacted with a wide range of locally native organisms. As a common factor among all those locations, this globetrotter ladybird makes for a great study subject on global ecological interactions.

AUTHOR CONTRIBUTIONS

DH: conceptualization. MG: writing—original draft and visualization. MG and DH: writing—review and editing. Both authors have read and agreed to the published version of the manuscript.

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Do Biotic and Abiotic Factors Influence the Prevalence of a Common Parasite of the Invasive Alien Ladybird *Harmonia axyridis*?

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Hesperomyces virescens (Ascomycota, Laboulbeniales), a fungal ectoparasite, is thus far reported on *Harmonia axyridis* from five continents: North and South America, Europe, Africa, and Asia. While it is known that *He. virescens* can cause mortality of *Ha. axyridis* under laboratory conditions, the role of biotic and abiotic factors in influencing the distribution of *He. virescens* in the field is unknown. We collected and screened 3,568 adult *Ha. axyridis* from 23 locations in seven countries in Central Europe between October and November 2018 to test the effect of selected host characters and climate and landscape variables on the infection probability with *He. virescens*. Mean parasite prevalence of *He. virescens* on *Ha. axyridis* was 17.9%, ranging among samples from 0 to 46.4%. Host sex, climate, and landscape composition did not have any significant effect on the infection probability of *He. virescens* on *Ha. axyridis*. Two color forms, f. *conspicua* and f. *spectabilis*, had a significantly lower parasite prevalence compared to the common *Ha. axyridis* f. *novemdecimsignata*.

Keywords: community ecology, *Hesperomyces*, Laboulbeniales, parasite prevalence, precipitation, temperature, spatial modeling, agricultural landscape

INTRODUCTION

Parasites may be the least studied life form on the planet (Price, 1980; Windsor, 1990, 1995). In their call for a "global parasite conservation plan," Carlson et al. (2020) proposed 12 major goals within four themes. These themes are data collection and synthesis (aimed at describing parasites and incorporating them into biodiversity surveys, among others), risk assessment and prioritization (documenting drivers of parasite declines and develop regional and global Red

Lists), conservation practice (e.g., building parasite conservation capacity), and outreach and education. One of the most common parasites of the globally invasive harlequin ladybird *Harmonia axyridis* (Coleoptera, Coccinellidae) is *Hesperomyces virescens*, which was for the first time observed on this host in 2002 (Garcés and Williams, 2004).

Hesperomyces virescens (Ascomycota, Laboulbeniales) is a biotrophic fungus that has a very wide distribution with confirmed reports in North and South America, Europe, Africa, and Asia (Haelewaters et al., 2017). Based on the results of an integrative taxonomic approach, we know that *He. virescens* is a complex of multiple species segregated by hosts (Haelewaters et al., 2018a). Thus far, two species have been formally described in the complex, *He. halyziae* (Haelewaters and De Kesel, 2020) and *He. parexochomi* (Crous et al., 2021). The other species, including the one associated with *Ha. axyridis*, are awaiting formal description. The parasite prevalence of *He. virescens* on *Ha. axyridis* differs among geographic regions and, exceptionally, may be as high as 96.5% in a given ladybird population, as reported in Meise, Belgium (February 2012, $n = 107$) and in Westmoreland, New Hampshire, United States (December 2012; $n = 83$) (Haelewaters et al., 2017). As a result, *He. virescens* has recently gained traction among entomologists as a potential biological control agent against *Ha. axyridis*.

Little information is available about the ecology of *He. virescens*. A recent experimental study demonstrated that infection with *He. virescens* affects the survival of *Ha. axyridis* on its own and when ladybirds are co-infected with either of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium brunneum* (Haelewaters et al., 2020). Thus far, however, potential effects of biotic and abiotic factors on the distribution of *He. virescens* and its parasite prevalence on *Ha. axyridis* are unknown. This information is fundamental for studies in conservation, applied ecology, and biocontrol strategies (Ferrier, 2002; Rushton et al., 2004; Magan, 2021). In this study, we collected adult specimens of *Ha. axyridis* across Central Europe and evaluated how selected host traits and climate and landscape variables affect infection patterns with *He. virescens*. Variables tested included host sex, host color form, color of elytra, proportion of agricultural and forested areas (European Environment Agency, 2020), temperature, and precipitation (Fick and Hijmans, 2017).

MATERIALS AND METHODS

Ladybirds were collected either by hand or using a mouth-operated aspirator from October to November 2018 in different Central European countries (**Figure 1**): Croatia (Istria County), the Czech Republic (Central Bohemian Region, South Bohemian Region, Plzeň Region), Germany (State of Bavaria), Hungary (Hajdú-Bihar County), Poland (Mazovian Voivodeship), Slovakia (Nitra Region), and Switzerland (Canton of Jura). Contributors were asked to collect at least 100 specimens from each ladybird population. Geographic coordinates were recorded and can be found in **Supplementary File 1**. Specimens were preserved in 70% ethanol until examination in the laboratory.

Ladybirds were screened under 40–50× magnification for the presence of non-hyphal thalli of *He. virescens* (sensu De Kesel, 2011; Haelewaters et al., 2018a). For each ladybird, the following traits were recorded: sex (see McCornack et al., 2007); color form [non-melanic f. *novemdecimsignata* (also referred to as *succinea*), and melanic f. *conspicua*, f. *axyridis*, and f. *spectabilis*]; color of elytra (for non-melanics) or spots (for melanics) (yellow, orange, red; Fiedler and Nedvěd, 2019). For *novemdecimsignata* specimens, we described whether spots were well-circumscribed (0), missing or fewer in number than typically present (−), or large and touching each other (+) (Fiedler and Nedvěd, 2019). For each population, no matter the number of sampled ladybirds, we screened 100 randomly selected specimens. When available, we screened and processed additional specimens of the melanic forms to avoid statistical restrictions due to these forming in low percentages. Screening results for all processed ladybirds are available in **Supplementary File 1**. After processing, voucher specimens were deposited in the Purdue Entomology Research Collection (West Lafayette, IN, United States) under the following accession numbers: PERC 0147670–0147680.

All statistical analyses were performed using the R software, version 3.6.3 (R Core Team, 2020). To identify the variables influencing the infection probability of *He. virescens* on *Ha. axyridis*, we used generalized mixed effect models (GMEM) with a binomial data distribution (infected yes/no) [function glmer(), R package *lme4*; Bates et al., 2015]. We included host sex, host color form, color of elytra, the proportion of agricultural, and forested areas in a buffer surrounding each sampling location (100 m, 300 m, 600 m, 1 km, and 2.5 km), and climate variables. Urban area was excluded from the analysis because it was highly collinear with the other predictor variables. For each buffer radius, a separate model was calculated, resulting in five distinct models. The landscape variables were obtained by extracting the landscape composition of Copernicus Corine Land Cover images taken in 2018 (European Environment Agency, 2020) with the help of R package *raster*, using the function extract (Hijmans et al., 2020). We pooled values in the categories "broad-leaved forest," "coniferous forest," and "mixed forest" into the forested area variable, whereas the agricultural area variable consisted of values for the categories "non-irrigated arable land," "fruit trees and berry plantations," "pastures," "complex cultivation patterns," and "land principally occupied by agriculture, with significant areas of natural vegetation" (**Supplementary File 2**). We further calculated three models using a "ring buffer" or annulus (sensu Rey et al., 2020), one for each of the following radius combinations: 100 m inner and 300 m outer radius, 300 m inner and 600 m outer radius, and 600 m inner and 1 km outer radius.

Climate variables were extracted from WorldClim with a resolution of 30 arc seconds (ca. 1 × 1 km) (Fick and Hijmans, 2017) (**Supplementary File 2**). As variables for temperature and humidity are generally collinear, we decided to summarize the following variables using a principal component analysis (PCA) [prcomp(), R package *stats*; R Core Team, 2020]: annual mean temperature, mean maximum temperature in the hottest month, mean minimum temperature in the coldest month,

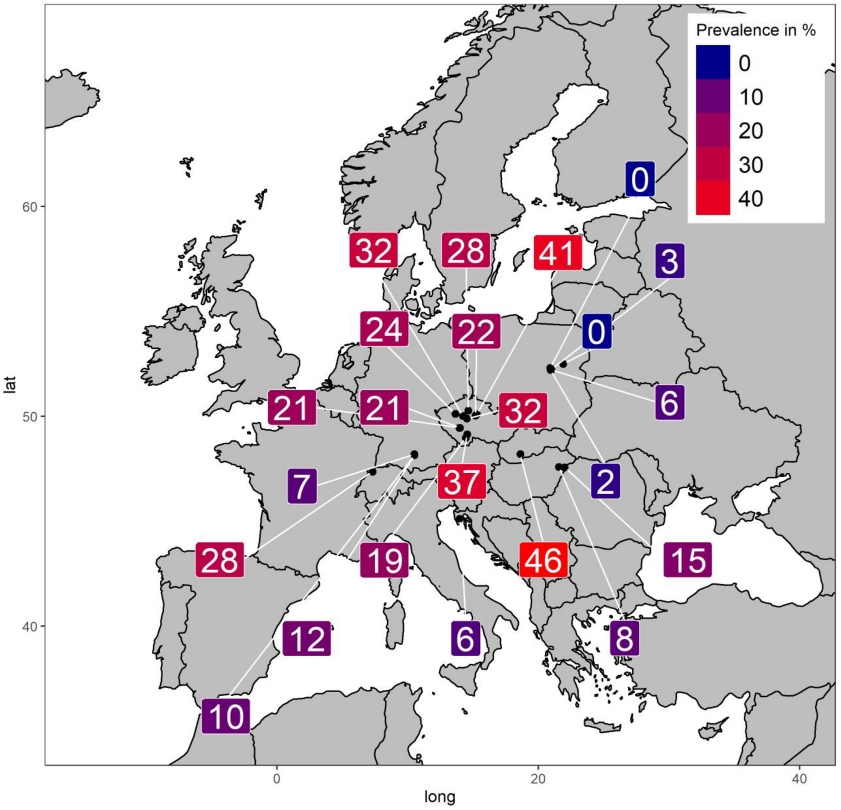

FIGURE 1 | Parasite prevalence by sampled population.

annual precipitation, precipitation during the wettest month, and precipitation during the driest month. We extracted the values for the first two dimensions for all sampling sites (**Supplementary File 2**). Statistical processing revealed two principal dimensions that explained 64 and 30.1% of the climatic variation, respectively. Finally, we included the random intercept *collection* nested within *sampling region* to address repeated sampling at the same location and also spatial autocorrelation as suggested by Zuur et al. (2010).

Hypothesis testing was done using likelihood ratio tests, with p values calculated based on χ^2 distributions, declaring an effect significant when $p \leq 0.05$. Nine models were compared, namely, the Null Model and the model with variables of interest within the different buffer radii (100 m, 300 m, 600 m, 1 km, and 2.5 km) and annulus radii (100–300 m, 300–600 m, and 600 m–1 km). Model selection happened using the Akaike Information Criterion (Akaike, 1974). For all models, we calculated pseudo-R^2 values to estimate model fit by accounting for the variation explained by both fixed and random effects [function r2(), R package *performance*; Lüdecke et al., 2020].

RESULTS

We screened a total of 3,568 ladybirds, resulting in a mean infection prevalence of 17.9%, ranging from 0 to 46.4% among sampled populations (**Figure 1**). The population with the highest prevalence of *He. virescens* was from Levice in southwestern Slovakia. Two populations showed no visible signs of *He. virescens* infection, both of which were from Poland. Forty-seven individuals were excluded from statistical analyses, including a single f. *intermedia* specimen from Warsaw, Poland and 46 specimens with missing information on elytral color. Likelihood ratio tests confirmed that each of the eight models explained the observed variance better than chance (**Table 1**), while the conditional pseudo R^2-values estimated model fit at around 0.33, indicating good fit (**Table 1**). We only found variables on host individual characters to have significant effects on the parasite prevalence of *He. virescens* on *Ha. axyridis* (**Tables 2**, **3**). The significant effect of color form was consistent in all five models, with the color forms f. *conspicua* and f. *spectabilis* being less often infected compared to the common form f. *novemdecimsignata*(0). There was a trend for f. *novemdecimsignata*(-) to be less likely infected by *He. virescens* compared to the common form f. *novemdecimsignata*(0). This trend was consistent over all candidate models but not significant. Finally, the color of elytra had a significant effect on the infection probability, with individuals with red elytra being more likely and individuals with yellow elytra less likely infected compared to individuals with orange elytra. Host sex, climate, and habitat composition resulted in not having any significant effect on the infection probability of *He. virescens* on *Ha. axyridis*.

TABLE 1 | Results of model (dependency of *Hesperomyces virescens* prevalence on *Harmonia axyridis* on coloration and climate and habitat properties) evaluation showing candidate models being significantly better than the null model and estimated pseudo-R^2 values indicating model fit.

Model	AIC	χ^2	*p* value	pseudo-R^2
Null Model	2,934.0			
Buffer 100 m	2,880.7	77.3	<0.001***	0.35
Buffer 300 m	2,880.1	77.9	<0.001***	0.33
Buffer 600 m	2,879.3	78.6	<0.001***	0.32
Buffer 1 km	2,879.6	78.4	<0.001***	0.32
Buffer 2.5 km	2,880.8	77.2	<0.001***	0.35
Annulus 100–300 m	2,880.1	77.9	<0.001***	NA
Annulus 300–600 m	2,879.2	78.8	<0.001***	0.32
Annulus 600 m–1 km	2,879.8	78.2	<0.001***	0.33

*Significance levels at: ***p < 0.001.*

DISCUSSION

Ha. axyridis–He. virescens

The first published record of *He. virescens* on *Ha. axyridis* in Europe was made in the winter of 2006–2007, from Meise in Belgium (De Kesel, 2011). Other country records followed quickly, from the Netherlands (2008), Germany (2008–2009), Croatia and the Czech Republic (2013), Hungary and Poland (2014), Slovakia (2015), Bulgaria and Greece (2017), and most recently European Russia and Switzerland (2018) (Herz and Kleespies, 2012; Ceryngier and Twardowska, 2013; Ceryngier et al., 2013; Pfliegler, 2014; Gorczak et al., 2016; Ceryngier and Romanowski, 2017; Haelewaters et al., 2017; van Wielink, 2017; Orlova-Bienkowskaja et al., 2018; this paper). Many of these papers show that the parasite prevalence of *He. virescens* differs significantly over time and in space (Raak-van den Berg et al., 2014; Haelewaters et al., 2017). Differences in Laboulbeniales prevalence among locations have been attributed to host population density and habitat type (Scheloske, 1969; De Kesel, 1996), but to date, no data were thus far available with regard to the *He. virescens–Ha. axyridis* association.

Effect of Biotic Factors

A remarkable finding from our study is that the melanic color forms f. *conspicua* and f. *spectabilis* were less often infected with *He. virescens* compared to the common nineteen-spotted f. *novemdecimsignata*(0). Two other studies investigated the relationship between the degree of melanization and infection patterns. Haelewaters et al. (2018b) observed a slight trend to higher intensity of parasitism in more melanic males of *Ha. axyridis* f. *novemdecimsignata*. Fiedler and Nedvěd (2019) found (i) a positive association between putative age groups of *Ha. axyridis* estimated as carotenoid content and infection with *He. virescens* and (ii) a negative association between elytral melanization of *Ha. axyridis* f. *novemdecimsignata* specimens and infection. The latter was explained by the fact that younger ladybirds emerged later in the year, with lower temperatures inducing extensive melanization; since they were younger, they had less opportunities to be parasitized by *He. virescens*. Our results are in line with the findings of Fiedler and Nedvěd (2019), but a definitive answer as to how elytral melanization affects the susceptibility to infection with *He. virescens* remains unclear. The black coloration of melanic forms of *Ha. axyridis* is negatively correlated with the total content of alkaloids (Bezzerides et al., 2007), which serve as defense against predators and pathogens (Röhrich et al., 2011). Then, heavier melanization should result in more infection with *He. virescens*, but our results are inconsistent with this hypothesis. Future work—performing bioassays in controlled settings and analyzing expression levels of immune genes—is needed to shed light on the susceptibility of different color morphs to *He. virescens*.

The results of our modeling approach show a significant correlation between elytral color and the infection probability of *Ha. axyridis* with *He. virescens*. Carotenoid accumulation, and thus red color intensity, is a function of ladybird age (Bezzerides et al., 2007; Nedvěd et al., 2019). In our study, older individuals of *Ha. axyridis* accumulated more *He. virescens* inoculum. Similar observations were made by Fiedler and Nedvěd (2019), particularly that individuals with red elytra are more likely to be infected. *Hesperomyces virescens* transmits among ladybirds through physical contacts (during mating and in overwintering aggregations), but auto-transmission by grooming or cleaning also occurs. These factors contribute to parasite prevalence (at the level of population) and thallus density (at the individual level) being positively correlated with host age (Riddick and Schaefer, 2005; Nalepa and Weir, 2007; Haelewaters et al., 2017) and thus, incidentally, with elytral color.

Effect of Abiotic Factors

Mean parasite prevalence did not significantly change according to any of the temperature variables tested [Kruskal–Wallis test, function kruskal.test(), R package stats; R Core Team, 2020], but our sampling scheme was somewhat limited, with only localities in Central European countries. For a broader understanding of the associations between *He. virescens* and *Ha. axyridis* within Europe, we recommend incorporation of data from northern and southern European countries in order to compile a dataset with a larger range in temperature. Collections also need to be expanded to city centers (e.g., in parks, community gardens, edges of playgrounds). A negative correlation was found between parasite prevalence of *He. virescens* sensu lato on *Adalia bipunctata* and distance from the city center of London (Welch et al., 2001). Prevalence in central London was as high as 40% (n = 105), whereas it was 0% outside of the urban area at a distance of 25 km. This could be linked to increased temperatures in urban environments (urban heat island effect), but this has not yet been tested. Adriaens et al. (2008) highlighted the idea that *Ha. axyridis* is less frequently found in natural landscapes compared to more urbanized and anthropogenic landscapes. Habitat preference may also be an important factor in the parasitism with *He. virescens*. Factors promoting *Ha. axyridis* may indirectly promote *He. virescens*. Particularly, a higher dominance of *Ha. axyridis* in a given ladybird community will be beneficial for ascospore transmission among individuals. The number of generations of *Ha. axyridis* in Central Europe varies from two to three and is probably dependent on habitat summer temperatures

TABLE 2 | Obtained parameters of each candidate model addressing the prevalence of infection by *Hesperomyces virescens* for different buffer radii (100 m, 300 m, 600 m, 1 km, 2.5 km).

	100 m buffer					**300 m buffer**				
	Estimate	**Std. Error**	**z value**	***p* value**		**Estimate**	**Std. Error**	**z value**	***p* value**	
(Intercept)	−1.597	0.507	−3.149	0.002	**	−1.652	0.480	−3.441	0.001	***
f. *axyridis*	0.299	0.768	0.389	0.697		0.303	0.769	0.394	0.693	
f. *conspicua*	−0.944	0.282	−3.347	0.001	***	−0.942	0.282	−3.344	0.001	***
f. *spectabilis*	−0.469	0.156	−2.996	0.003	**	−0.469	0.156	−2.999	0.003	**
f. *novemdecimsignata*(-)	−0.224	0.122	−1.846	0.065	.	−0.225	0.121	−1.853	0.064	.
f. *novemdecimsignata*(+)	0.020	0.170	0.117	0.907		0.022	0.170	0.128	0.898	
red	0.599	0.124	4.839	0.000	***	0.594	0.124	4.797	0.000	***
yellow	−0.663	0.154	−4.310	0.000	***	−0.665	0.154	−4.318	0.000	***
sex m	−0.003	0.102	−0.032	0.975		−0.003	0.102	−0.030	0.976	
agricultural	0.036	0.123	0.293	0.769		0.090	0.108	0.835	0.403	
forest	0.029	0.097	0.304	0.761		0.044	0.096	0.453	0.651	
Dim1	0.327	0.210	1.556	0.120		0.279	0.205	1.360	0.174	
Dim2	−0.090	0.127	−0.713	0.476		−0.100	0.122	−0.822	0.411	
	600 m buffer					**1 km buffer**				
	Estimate	**Std. Error**	**z value**	***p* value**		**Estimate**	**Std. Error**	**z value**	***p* value**	
(Intercept)	−1.679	0.461	−3.644	0.000	***	−1.654	0.466	−3.549	0.000	***
f. *axyridis*	0.303	0.768	0.395	0.693		0.302	0.768	0.393	0.694	
f. *conspicua*	−0.944	0.282	−3.352	0.001	***	−0.948	0.282	−3.364	0.001	***
f. *spectabilis*	−0.469	0.156	−3.005	0.003	**	−0.471	0.156	−3.017	0.003	**
f. *novemdecimsignata*(-)	−0.223	0.121	−1.839	0.066	.	−0.221	0.121	−1.824	0.068	.
f. *novemdecimsignata*(+)	0.023	0.170	0.136	0.892		0.023	0.170	0.133	0.894	
red	0.591	0.124	4.771	0.000	***	0.593	0.124	4.780	0.000	***
yellow	−0.666	0.154	−4.331	0.000	***	−0.668	0.154	−4.341	0.000	***
sex m	−0.002	0.101	−0.018	0.985		−0.002	0.101	−0.016	0.987	
agricultural	0.135	0.105	1.291	0.197		0.123	0.104	1.178	0.239	
forest	0.039	0.090	0.432	0.666		0.027	0.088	0.304	0.761	
Dim1	0.248	0.198	1.249	0.212		0.264	0.200	1.321	0.187	
Dim2	−0.113	0.120	−0.946	0.344		−0.120	0.122	−0.981	0.327	
	2.5 km buffer									
	Estimate	**Std. Error**	**z value**	***p* value**						
(Intercept)	−1.571	0.502	−3.129	0.002	**					
f. *axyridis*	0.300	0.769	0.390	0.697						
f. *conspicua*	−0.946	0.282	−3.356	0.001	***					
f. *spectabilis*	−0.470	0.156	−3.005	0.003	**					
f. *novemdecimsignata*(-)	−0.224	0.121	−1.841	0.066	.					
f. *novemdecimsignata*(+)	0.021	0.170	0.122	0.903						
red	0.598	0.124	4.808	0.000	***					
yellow	−0.665	0.154	−4.319	0.000	***					
Sex m	−0.002	0.101	−0.020	0.984						
Agricultural	0.030	0.157	0.189	0.850						
Forest	0.034	0.113	0.299	0.765						
Dim1	0.334	0.216	1.544	0.123						
Dim2	−0.086	0.136	−0.634	0.526						

Significance levels at: $\cdot p < 0.1$, $^{**}p < 0.01$, $^{***}p < 0.001$.

and prey availability. Population densities can increase due to the use of various prey patches enabled by high mobility in combination with the weak tendency for diapause extending the breeding period (Honek et al., 2018). In urbanized areas, the breeding season of *Ha. axyridis* is advanced by 2–3 weeks (Honek et al., 2021).

In addition to our buffer radius models, which are often used for forest management on a landscape scale (Brouwers et al., 2010), we also employed annulus radii to better model the behavior of *Ha. axyridis* in autumn. Our sampling localities are not where ladybirds became infected with *He. virescens*. As a result, the habitat variables might not be accurate for the habitats

TABLE 3 | Obtained parameters of each candidate model addressing the prevalence of infection by *Hesperomyces virescens* for different annulus radii (100–300 m, 300–600 m, 600 m–1 km).

	100–300 m					300–600 m					600 m–1 km				
	Estimate	**Std. Error**	**z value**	***p* value**		**Estimate**	**Std. Error**	**z value**	***p* value**		**Estimate**	**Std. Error**	**z value**	***p* value**	
(Intercept)	−1.651	0.478	−3.454	0.001	***	−1.671	0.460	−3.636	0.000	***	−1.634	0.472	−3.464	0.001	***
f. *axyridis*	0.304	0.769	0.395	0.693		0.302	0.768	0.393	0.694		0.301	0.768	0.393	0.695	
f. *conspicua*	−0.943	0.282	−3.346	0.001	***	−0.946	0.282	−3.357	0.001	***	−0.949	0.282	−3.369	0.001	***
f. *spectabilis*	−0.469	0.156	−3.001	0.003	**	−0.470	0.156	−3.010	0.003	**	−0.472	0.156	−3.021	0.003	**
f. *novemdecimsignata*(-)	−0.225	0.121	−1.853	0.064	.	−0.222	0.121	−1.832	0.067	.	−0.221	0.121	−1.820	0.069	.
f. *novemdecimsignata*(+)	0.022	0.170	0.129	0.898		0.023	0.170	0.135	0.892		0.022	0.170	0.130	0.897	
red	0.594	0.124	4.795	0.000	***	0.591	0.124	4.773	0.000	***	0.594	0.124	4.789	0.000	***
yellow	−0.665	0.154	−4.320	0.000	***	−0.667	0.154	−4.333	0.000	***	−0.668	0.154	−4.341	0.000	***
sex m	−0.003	0.101	−0.030	0.976		−0.002	0.102	−0.015	0.988		−0.002	0.101	−0.016	0.987	
agricultural	0.093	0.106	0.877	0.381		0.139	0.104	1.336	0.181		0.111	0.105	1.058	0.290	
forest	0.046	0.097	0.472	0.637		0.037	0.089	0.419	0.675		0.024	0.087	0.269	0.788	
Dim1	0.275	0.204	1.348	0.178		0.249	0.197	1.262	0.207		0.279	0.201	1.388	0.165	
Dim2	−0.101	0.121	−0.829	0.407		−0.114	0.119	−0.957	0.338		−0.119	0.124	−0.964	0.335	

Significance levels at: $\cdot p < 0.1$, $^{**}p < 0.01$, $^{***}p < 0.001$.

that the ladybirds occupied when they became infected with the fungus. Generally, we estimate that *Ha. axyridis* ladybirds fly 500 m during autumn migration. Detailed observations of ladybirds in České Budějovice, Czech Republic allowed the exact measurement of migration distance from before flight and after flight, which was 200–500 m toward the north (O. Nedvěd, unpublished). The longest flights measured were around 1,800 m in laboratory flight mills (Růžička, 1984). When Nalepa et al. (2005) conducted their experiments to test the role of visual contrast in autumn behavior of *Ha. axyridis* in 4 ha of open pasture, the required flight was about 200 m (Nalepa et al., 2005). In open fields in Japan, the median flight distance was around 400 m (Seko et al., 2008). We tested three different annulus radii (100–300 m, 300–600 m, and 600 m–1 km), but the results of our candidate models were highly similar to the buffer radius models. Aggregations of ladybirds are often formed on walls of building oriented toward the south or west (Kidd et al., 1995; Raak-van den Berg et al., 2012; Haelewaters et al., 2018b). As a result, we suggest that the annulus modeling approach could be made even more specific by obtaining landscape variables for an annulus section (or ring pie chart) facing the direction where ladybirds likely migrated from. This is methodologically complex and out of the scope of this paper, but is a consideration for future studies.

Community ecology research of Laboulbeniales is still in its infancy, with thus far only two published studies. Szentiványi et al. (2019) investigated whether climatic variables (temperature, humidity) influenced the distribution of ant- and bat fly-associated Laboulbeniales. They found that both the presence and prevalence of Laboulbeniales on their hosts were positively associated with low annual mean temperature and humidity. In addition, based on the study of more than 9,374 workers of the invasive ant *Lasius neglectus* in 66 colonies, Gippet et al. (2021) found that the presence of *Laboulbenia formicarum* on the ants was positively linked to warmer and dryer conditions at lower elevations. These are seemingly contrary results, and our data render drawing general conclusions for these microfungi even more complex. However, the direction of the effects of some of these variables may be species-specific, as suggested by Dumolein (2021). One could make the case that the combined analysis of presence/absence data in Szentiványi et al. (2019) may obscure true interactions, and thus that separate analyses would give a more accurate picture of how bioclimatic variables affect the distribution of the two assessed study systems—the bat fly-associated *Arthrorhynchus* spp. vs. the ant-associated *Rickia wasmannii*. We note that the studies of Szentiványi et al. (2019) and Gippet et al. (2021) used outside climatic data as obtained from MERRAclim, but many of these ant–Laboulbeniales and bat fly–Laboulbeniales interactions and the host dynamics resulting in fungal transmission take place in ant nests and bat roosts, respectively. Ant nest and bat roosting environments are characterized by their own microclimatic conditions, which likely play a role in shaping the distribution of these species of Laboulbeniales. Efforts should be redirected at collecting temperature and relative humidity data (e.g., through automated readers) within these environments to test for the effect of these microclimate-specific abiotic traits on parasitism with Laboulbeniales.

Our results are the first for the *Ha. axyridis*–*He. virescens* study system based on specimens collected during autumn migration. Understanding the factors influencing the infection of Laboulbeniales on invasive ladybirds—including climatic and landscape variables as well as seasonality and host behavior (e.g., Raak-van den Berg et al., 2014; Haelewaters et al., 2015, 2017; Markó et al., 2016)—will help understand their global spread as they cross many different ecosystems and environmental conditions. Resolving this question will also inform potential biocontrol strategies because it will inform us under which conditions *He. virescens* may (or may not) thrive. The collection of standardized multi-year, multi-site field data will help in this regard in addition to controlled laboratory experiments.

DATA AVAILABILITY STATEMENT

The original data from this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

DH and ON designed the study. DH, TH, PC, RE, MG, KK, MK, NL, WPP, PZ, and ON collected the data. DH, TH, PC, and MH performed data analysis. DH, MCA, and ON acquired funding. DH and TH drafted the manuscript. DH revised the manuscript. All authors edited and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.773423/full#supplementary-material

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Ladybird communities in rural woodlands: Does an invader dominate?

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The invasive alien species *Harmonia axyridis* (Coleoptera: Coccinellidae) was first observed in the UK in 2004. Previous studies have demonstrated the adverse effects on other species of *H. axyridis* during its early stages of establishment. However, habitat factors are important in determining distribution and population trends of ladybirds. Whilst the abundance of *H. axyridis* is well known in the UK within urban and other managed habitats, much less is known about its abundance in the wider countryside. Here we present the results of surveys from rural woodland habitats to assess whether or not *H. axyridis* dominates coccinellid communities in these rural habitats. Additionally, we explored the relationship between coccinellid and aphid abundance within these habitats. All field sites were in Cambridgeshire or Suffolk, East Anglia, UK and were surveyed between May and October 2016 and 2017. Three deciduous sites and three coniferous sites were included in the study. Surveys were conducted using a standardised approach involving sweep-netting within grass margins and tree beating to sample ladybirds from trees. Three distinct vegetation structures or layers were surveyed within both the coniferous and deciduous sites; tree, shrub and herb layer. All captured coccinellids were identified to species-level. Seventeen species of coccinellid and over 1300 individuals were recorded during the study period from two distinct site types (deciduous, coniferous). Species richness was lower at deciduous sites (n = 12) in comparison to coniferous (n = 16) sites. The coccinellid community also did not appear to be dominated by *H. axyridis* at rural sites, in contrast to urban areas. Deciduous woodland appeared to be a lesser preferred habitat of *H. axyridis* than coniferous woodland. Additionally, there was a distinct difference in the coccinellid community in relation to vegetation structure (across the tree, shrub and herb layers) between coniferous and deciduous sites. Our results indicate that there appear to be distinct native coccinellid communities at deciduous and coniferous sites. We discuss the way in which rural woodlands could act as a refuge for some native coccinellids.

KEYWORDS

biological invasions, Coccinellidae, *Harmonia axyridis*, invasive alien species, ladybirds, non-native species, rural habitat, woodland

Introduction

The natural world is changing rapidly with increased human activity having dramatic consequences for biodiversity and ecosystems (IPBES, 2019). The last 50 years has seen a rapid escalation in the movement of animals and plants, locally and globally, often resulting in species establishing in habitats where they would not otherwise naturally occur (Blackburn et al., 2014; Lucy et al., 2016). The number of species being introduced to regions beyond their natural range has risen steadily over the last two centuries, with increasing trade and travel but also linked to other drivers of environmental change that facilitate biological invasions such as land and sea use change, climate change and pollution (Seebens et al., 2017; IPBES, 2019). Change in invertebrate biodiversity, including the addition of invasive alien species within ecological networks, is a major concern globally (Didham et al., 2005; Mikanowski, 2017). When introduced to a new region, generalist alien species are more likely to become invasive than specialist species; this can result in native specialist species being outcompeted and thereby leading to functional homogenisation (Clavel et al., 2011). It can be difficult to ascertain the effect that the presence of an invasive alien species (IAS) may have on ecosystem function, however in recent years, there has been evidence of the effects on the invaded ecosystem and community (Simberloff et al., 2013) due to invasive alien plants (Liao et al., 2007) and invasive alien aquatic invertebrates (Crawford et al., 2006; Constable and Birkby, 2016; Mathers et al., 2016).

Many coccinellid species provide ecosystem services in the form of pest control (Roy et al., 2012; Honěk et al., 2017). While there have been notable successes of introducing coccinellids for biological control (Dixon et al., 1997; Fowler, 2004), a number of introduced species (e.g. *Harmonia axyridis, Coccinella septempunctata*) have become established beyond their release sites and have subsequently had negative effects on native coccinellid species (Evans, 2000; Adriaens et al., 2008; Brown et al., 2011a; Roy et al., 2016; Sloggett, 2017); hence considered as IAS. These IAS are often studied in urban (Brown et al., 2011a; Viglášová et al., 2017) or agricultural habitats (Bianchi et al., 2007; Grez et al., 2008; Grez et al., 2014a), however less is known of the ecology of these species in rural habitats[1].

In any habitat, a small number of dominant coccinellid species (between two and four) are expected to comprise around 90% of the community (Honěk, 2012). Selyemová et al. (2007) reported a diverse coccinellid community in rural coniferous woodland that was dominated by four species, however, *H. axyridis* was not established in the region at the time. When investigating overwintering coccinellids in coniferous woodland, Holecová et al. (2018) reported that *H. axyridis* was not the most abundant coccinellid. In the UK, just as *H. axyridis* was establishing, Brown et al. (2011a) reported that *H. axyridis* was largely absent from coniferous woodland. Furthermore, Purse et al. (2014) predicted coniferous woodland could be a refuge for native coccinellids because climate models suggest these habitats are suboptimal for *H. axyridis*. Vegetation structure of a habitat can also influence coccinellid assemblages. Grassland has been shown to be a refuge for native coccinellid species with very few invasive alien coccinellids recorded in this habitat (Diepenbrock and Finke, 2013). Rural woodland generally consists of a range of tree species and areas of wild herbs/grassland. In Michigan (USA) coccinellid species richness was higher when the habitat was more complex and contained a range of vegetation structures from deciduous trees to grassland and crops (Colunga-Garcia et al., 1997). When non-crop vegetation was added to an agricultural habitat, coccinellid abundance increased (Woltz and Landis, 2014) and intraguild predation between a native coccinellid and *H. axyridis* decreased (Amaral et al., 2015). Additionally, trees and grassland tend to have a more diverse coccinellid community than crops (Honěk, 2012). Beginning to understand how and why certain habitats are used by particular coccinellid species would be beneficial to understanding the relationship between *H. axyridis* and native specialist coccinellid species (Sloggett and Majerus, 2000a). Competition for food resources from *H. axyridis* is one of the reasons why native coccinellids may be negatively affected (Brown et al., 2011a) and so monitoring aphid abundance adds another dimension to studies of coccinellid community dynamics.

Harmonia axyridis is an intraguild predator; i.e. it preys upon other aphid natural enemies including the eggs and larvae of other coccinellid species (Roy et al., 2011; Brown et al., 2011a). It has been suggested that an ongoing increase of *H. axyridis* numbers may lead to the extinction of some coccinellid species locally (Harmon et al., 2007; Adriaens et al., 2010; Comont et al., 2014; Honěk et al., 2016). Harmon et al. (2007) highlighted the decline of *A. bipunctata* over a broad geographic range after the invasion of *C. septempunctata* and *H. axyridis* in the North America. The dramatic decline of *Coccinella novemnotata* in North America has also been attributed to a combination of pressures exerted by both *C. septempunctata* and *H. axyridis* (Losey et al., 2012b; Tumminello et al., 2015; Ducatti et al., 2017). In Europe there has been a decline in *A. bipunctata* (Belgium & UK), *Adalia decempunctata* and *Calvia quattuordecimguttata* (Czech Republic) since the arrival and establishment of *H. axyridis* (Brown et al., 2011a; Roy et al., 2012; Honěk et al., 2016; Brown and Roy, 2018). In the UK, distribution of *H. axyridis* is well known within urban and other anthropogenic habitats and Labrie et al. (2008) reported *H. axyridis* surviving very cold winters only where people dwell, as this species prefers to over-winter in anthropogenic structures. Additionally, Brown et al. (2011b) reported that *H. axyridis* tended to oviposit and feed at sites that have human structures nearby. However, much less is known on detailed habitat use of

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[1] In this study the term rural is used to describe the habitats surveyed that were based in woodland areas that were not within a town or city.

H. axyridis in the wider countryside (Brown et al., 2011a; Brown and Roy, 2018).

Considering the aforementioned studies together with the documented declines of native coccinellids in urban areas strongly correlated with the presence of *H. axyridis* in the UK (Roy et al., 2012), it is important to understand how native coccinellids are faring in rural areas (Viglášová et al., 2017). The aim of this study was to explore the relationship between the invasive alien *H. axyridis* and native coccinellid species in rural habitats.

Materials and methods

Field sites

All sites were in Cambridgeshire or Suffolk and were identified based on the presence of native tree species. During the 2016 field season, four deciduous sites (Brampton Wood, Monk's Wood, Raveley Wood and Wistow Wood) and two coniferous sites (two sites at King's Forest, a much larger woodland at approximately $23km^2$) were sampled. Wistow Wood was removed as a site for 2017 as there were not sufficient tree numbers or grassland habitat to complete full surveys. An additional coniferous site at King's Forest was added for surveying in 2017. Thus, during the 2017 field season three deciduous woodlands (Brampton Wood, Monk's Wood and Raveley Wood) and three coniferous woodlands (three sites at King's Forest) were surveyed (Table 1). Site locations can be found in Supplementary Material Figure S1. Grid references were recorded using a Garmin GPSmap 60CSx. Surveys took place from the beginning of May to the end of October incorporating two seasons: summer (May, June, July) and autumn (August, September, October). In order to standardise data collection sampling took place between 10:00 and 16:00 when weather conditions were favourable, i.e. when the temperature was greater than 14°C, conditions were dry and wind speeds were below 5 on the Beaufort scale (Jones et al., 2006). Some surveys were carried out when the temperature was below 14°C, however in such instances there was at least 60% sun. Humidity and ambient temperature were recorded using an EasyLog EL-21CFR-2-LCD.

Vegetation layers/structure

Three vegetation layers were selected for data collection; tree, shrub and herb layer. These layers encompass the key vegetation types found within a woodland and collectively contain the majority of UK ladybird species (Roy et al., 2013). The tree and shrub species selected for surveying were all native to the UK. Additionally, the number of individuals of each tree/shrub species was sufficient to allow regular visits during the sampling season at the respective woodland sites and avoid over-sampling. The herb layer (grassland/grass layer) comprised low vegetation including grasses, wildflowers, thistle, bramble, saplings etc. Vegetation height in the grass margins did not exceed one metre in height. The shrub layer (intermediate layer) comprised shrubs (Hall et al., 2004) and immature trees, with the sampled plants being no higher than three metres. The species selected for data collection were hazel (*Corylus avellana*) and hawthorn (*Crataegus monogyna*) in deciduous woodland and immature Scots Pine (*Pinus sylvestris*) and birch (*Betula pendula*) in coniferous woodland. The tree layer (mature layer) consisted of trees that were over three metres high with the target species being oak (*Quercus robur*) and field maple (*Acer campestre*) in deciduous woodland and mature Scots pine (*Pinus sylvestris*) and silver birch (*Betula pendula*) in coniferous woodland. The tree and shrub layer are on occasion referred to collectively as woodland and the herb layer referred to as grassland.

Survey methods

Sweep-netting was used to sample coccinellids in the herb layer. This method involved the use of a sweep net which comprised of a white canvas bag (46cm in diameter) attached to a metal ring on a large pole. One sweep was carried out for one metre of distance walked with 100 metres of grassland

TABLE 1 Locations with woodland type and year surveyed for each field site.

Site	Woodland Type	Grid References	County	2016 Surveys	2017 Surveys
Brampton Wood	Deciduous	TL1787 7018	Cambridgeshire	√	√
Monk's Wood	Deciduous	TL1976 8011	Cambridgeshire	√	√
Raveley Wood	Deciduous	TL2444 8184	Cambridgeshire	√	√
Wistow Wood	Deciduous	TL2963 8214	Cambridgeshire	√	X
King's Forest 01	Coniferous	TL8223 7374	Suffolk	√	√
King's Forest 02	Coniferous	TL8201 7417	Suffolk	√	√
King's Forest 03	Coniferous	TL8088 7153	Suffolk	X	√

being surveyed at deciduous and coniferous woodland sites only. Sweeping this area took approximately 25 minutes. An estimate of the percentage plant cover of the grass margin was determined by eye at each sampling point. Tree beating was used to collect ladybirds from the tree and shrub layers. This method involved using a stick (approximately 1.5 metres in length) to sharply tap tree branches whereby insects fell onto a large white beating tray (110cm x 86cm) (Roy et al., 2013). Three individual branches on each tree were sampled by tapping each branch three times in quick succession. Depending on accessibility, each survey was carried out around the full circumference of the tree. Ten trees of each species in both the intermediate and mature layers were surveyed in deciduous and coniferous woodland. Surveying ten trees within one gradient took approximately 25 minutes. All captured coccinellids were identified to species level in the field. Larvae in the early stages of development, especially first and second instar, are very difficult to identify to species level in the field and so where there was uncertainty the term 'Early Stage Larva' (ESL) was used. Additionally, third instar *Harmonia* spp. larvae are included in the ESL group due to the similarity between *H. axyridis* and *H. quadripunctata* at this life stage. Just over six percent of larval records were not identifiable due to their early stage (ESL) (Table S1) and were excluded from analyses. The number of aphids (adult and immature) captured during sweeping/tree beating were also recorded. Due to potentially very large numbers being present these numbers were estimated in increments of 5 (for example, 1, 5, 10, 15, 20, 25, etc.). Aphids (Aphidoidea) were identified to superfamily.

Data analysis

Analyses was carried out using R Studio (R Core Team, 2019) except for canonical correspondence analysis which was carried out in PAleontological Statistics (PAST) Version 3.23 (Hammer et al., 2001). The following R packages were used for basic analyses and visualisation of data: dplyr (Wickham et al., 2019), ggfortify (Horikoshi and Tang, 2016; Tang et al., 2016), ggplot2 (Wickham, 2016), ggpubr (Kassambara, 2018). For multivariate analyses three packages were used: Hotelling (Curran, 2018), lattice (Sarkar, 2008) and vegan (Oksanen et al., 2019). The remaining packages used for regression analyses were: fmsb (Nakazawa, 2018), lmtest (Zeileis and Hothorn, 2002), pscl (Zeileis et al., 2008), sandwich, (Zeileis, 2004; Zeileis, 2006), lattice and MASS (Venables and Ripley, 2002). Wilcoxon paired tests were used to compare abundances of native coccinellids and *H. axyridis* at the same locations, e.g. deciduous woodland. Spearman's correlation was utilised to investigate any association between both *H. axyridis* and native coccinellid abundance and that of aphids.

Regression models

Generalised linear models (GLM) were utilised to investigate the effects of site type (deciduous, coniferous), vegetation structure (tree, shrub, herb) and season (summer, autumn) on coccinellid and aphid abundance. Data from tree and shrub layer were analysed together under the collective term 'woodlands' when investigating site type while the grassland data was analysed separately due to differences in sampling method. Environmental variables (temperature, humidity) were included in the models. We assessed overdispersion commonly associated with count data and accordingly used log Likelihood, Akaike Information Criterion (AIC) and weighted AIC, with the weighted AIC being the deciding factor as to which model was the best fit. In the majority of cases, either a zero-inflated negative binomial (ZINB) model or negative binomial regression (NB) model were the best fit for the data, and on occasion the null model was the better fit. Temperature and humidity were checked for collinearity with a variance inflation factor (VIF). Neither variables were of concern with a VIF of < 1.2 each, and both were incorporated into the regression models. Table 2 presents which model was the best fit for explaining the effects of the variables on *H. axyridis*, native coccinellid and aphid abundance in rural woodlands and rural grasslands.

TABLE 2 Final model that was best fit when variables applied under the following conditions.

Location	Rural woodland	Deciduous woodland	Coniferous woodland
Dependent variable			
H. axyridis	Full ZINB	Full ZINB	Reduced NB
Native coccinellids	Reduced NB	Full ZINB	Reduced NB
Aphidoidea	Full ZINB	Full ZINB	Reduced NB
Location	**Rural grassland**	**Deciduous grassland**	**Coniferous grassland**
Dependent variable			
H. axyridis	N/A	N/A	N/A
Native coccinellids	Reduced NB	Null model	Full ZINB
Aphidoidea	Full ZINB	Full ZINB	Full NB

ZINB, Zero-inflated negative binomial; NB, Negative binomial; N/A, not applicable due not enough data available for analysis.

Model comparisons can be found in Supplementary Material, Tables S3–S19.

Diversity

Shannon Diversity was calculated for rural sites only and for native coccinellid species only. Simpson's diversity was not carried out as rare species or those recorded in low numbers are not given the same consideration by the index as more abundant species by this measure (Magurran, 2004; Morris et al., 2014). Differences in diversity across site types and season were calculated using t-tests while ANOVA was used to assess any differences in diversity within the vegetation structure followed by a *post-hoc* Tukey if any significances were apparent. Regression models were run to determine if native coccinellid diversity had any effect on the abundance of *H. axyridis* and native coccinellids.

Ordination

Canonical Correspondence Analysis (CCA) detects patterns of variation in a given community that can be explained by environmental data. The analysis focuses on beta-diversity (how dissimilar sites are) instead of alpha diversity (diversity of a site) (Zuur et al., 2007). This method of multivariate analysis generates an ordination diagram where a given species point is at the weighted average or centroid of the sites where it was recorded (ter Braak and Verdonschot, 1995). The qualitative environmental variables (site type and vegetation layer) are illustrated by a point that is the centroid of site points belonging to that group, for example the weighted average of the tree layer where the weight is the total abundance of the tree layer (ter Braak and Verdonschot, 1995). This analysis was used to investigate the relationship that two variables had on the coccinellid assemblage; site type (deciduous or coniferous) and vegetation layer (tree, shrub or herb). The coccinellid data were fourth root transformed to remove any effect of highly abundant species (Chessman, 2003; Pickwell, 2012). Interpretation of the resulting ordination is based on the eigenvalues, statistical significance determined by Monte Carlo permutation test and ecological interpretability (ter Braak and Verdonschot, 1995). In this case, the biplot rule (described below) was applied as the eigenvalues were less than 0.4 and this rule is more informative than the centroid rule when eigenvalues are low (ter Braak and Verdonschot, 1995). Firstly, the direction of maximum change in the relative abundance of a species (e.g. species X or Y) was determined by drawing a line from Species X to the origin. Subsequently the sites were then projected onto the arrow for Species X, illustrating the share each site (site A or B) has in the total abundance of each species (ter Braak and Verdonschot, 1995; Zuur et al., 2007). To interpret how a species relates to an environmental variable, imagine the variable line (e.g. 'Type') is extended in the opposite direction for the same distance, forming an axis of its own. Each species can be projected perpendicular to the axis, indicating the species relationship with that variable (Zuur et al., 2007). The combination of regression models, the Shannon diversity index and the ordination analysis yielded a detailed representation of coccinellid assemblages.

Results

Seventeen species of coccinellid totalling 1,330 individuals were recorded during the study period across three different vegetation gradients (tree, shrub & herb layer) from deciduous and coniferous woodland. Just eight of these coccinellid species were recorded in the herb layer in comparison to 16 species on trees & shrubs in woodland (see Supplementary Material Tables S1, S2). Five species (*Myzia oblongoguttata, Myrrha octodecimguttata*, *Scymnus suturalis*, *Subcoccinella vigintiquattuorpunctata* and *Tytthaspis sedecimpunctata*) were recorded in coniferous woodland only, while one species (*Psyllobora vigintiduopunctata*) was recorded in deciduous woodland only. In grassland, four coccinellid species (*Exochomus quadripustulatus*, *S. suturalis*, *S. vigintiquattuorpunctata* and *T. sedecimpunctata*) were recorded at coniferous sites only. Inclusive of all three vegetation layers, species richness was lower at deciduous (n = 12) than coniferous (n = 16) sites.

Coccinellids in tree & shrub layer

Rural woodland site type (deciduous and coniferous) was analysed separately. Native coccinellid abundance was significantly greater than that of *H. axyridis* in deciduous woodland (median = 2 and 0 respectively, Z = -5.43, p < 0.001, r = 0.60) and coniferous woodland (median = 3.5 and 1 respectively, Z = -4.15, p < 0.001, r = 0.50) (Figure 1). The binary model of the ZINB revealed that the likelihood of recording *H. axyridis* was higher in the summer rather than autumn across rural woodlands combined (z = -3.011, p = 0.003). The only variable that affected *H. axyridis* abundance was vegetation layer in both deciduous only and coniferous only woodland, with a greater number recorded in the tree layer (z = 2.65, p = 0.008 and z = 2.82, p = 0.005 respectively) (Figure 1). The abundance of *H. axyridis* was higher during the summer (z = 4.78, p < 0.001) in deciduous woodland with no effect of season apparent in coniferous woodland. In addition to the results from the logistic model, the binary model explained in greater detail what the zeros represented in these data, with the likelihood of recording *H. axyridis* being significantly higher in coniferous woodland in comparison to deciduous woodland (z = 3.67, p =

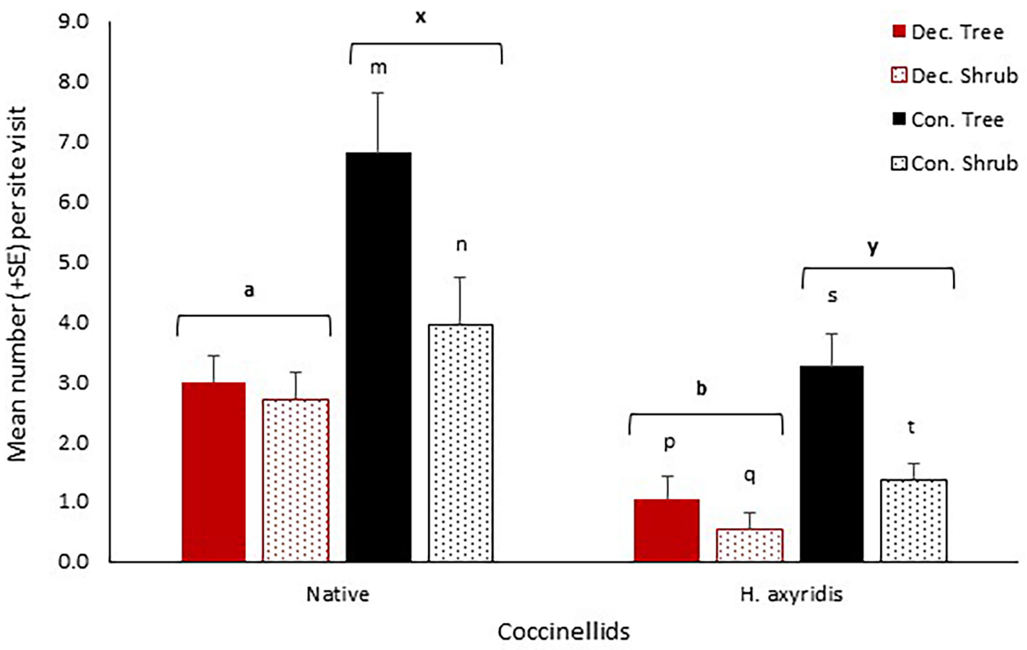

FIGURE 1
Mean number (+SE) per site visit of coccinellids recorded in woodland at deciduous-only and coniferous-only sites in Cambridgeshire and Suffolk. Native = all native coccinellids recorded; Dec. = Deciduous; Con. = Coniferous; Tree = Tree layer; Shrub = Shrub layer. Square brackets indicate the grouping of the Tree and Shrub layer for the respective deciduous and coniferous sites. Consecutive letters indicate where significant differences occur.

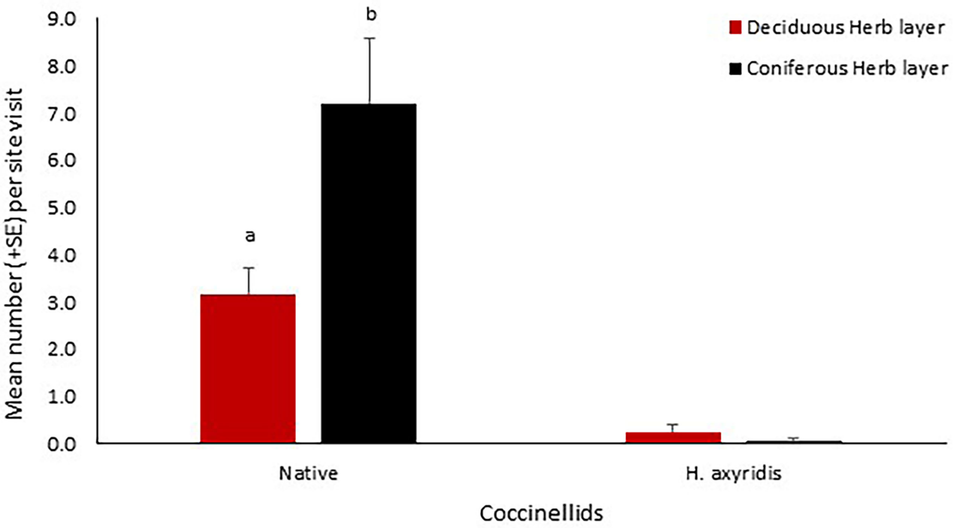

FIGURE 2
Mean number (+SE) per site visit of coccinellids recorded in the grass layer at deciduous and coniferous sites in Cambridgeshire and Suffolk. Native = all native coccinellids recorded. Consecutive letters indicate where significant differences occur.

0.0002). In rural woodland, deciduous sites had a significantly lower number of native coccinellids than did coniferous sites (z = -3.16, p = 0.002) (Figure 1). In deciduous woodland, vegetation layer had no effect on native coccinellid abundance, however, abundance was significantly higher in the tree layer of coniferous woodland as opposed to the shrub layer (z = 2.67, p = 0.008) (Figure 1). Season did not influence the abundance of native coccinellids in deciduous only or coniferous only woodland.

Coccinellids in herb layer

Eight coccinellid species totalling 405 individuals were recorded in the grassland habitat. Two of the species, *S. vigintiquattuorpunctata* and *T. sedecimpunctata* were predominantly confined to grassland (with only two occurrences of *S. vigintiquattuorpunctata* on trees). Very few *H. axyridis* were recorded in the herb layer (n = 12) and as a result it was not possible to apply any statistical analysis. Significantly fewer native coccinellids were recorded in grassland within deciduous habitat (z = -3.08, p = 0.002) as indicated by the reduced negative binomial model (Figure 2) than within grassland in coniferous habitats. In coniferous woodland, native coccinellids were significantly more abundant during summer rather than autumn (z = 3.23, p = 0.001). The null model was the best fit to investigate coccinellid abundance in grassland at deciduous sites revealing no effect of season on native coccinellid abundance.

Coccinellid diversity

When considered as an entire habitat, coniferous sites hosted a significantly higher native coccinellid diversity (t = 5.83, p < 0.001) than deciduous woodlands (Figure 3). In deciduous woodland sites, native coccinellid diversity varied significantly (one-way ANOVA: F = 4.35, p = 0.015) with the tree layer having greater diversity than the grass layer (p = 0.01) as revealed by a *post-hoc* Tukey test. Coniferous sites also exhibited differences between the different vegetation structures (F = 9.24, p <0.0002) with a significantly lower diversity in both the shrub layer and grass layer (p = 0.0005 & p = 0.001 respectively; Figure 3) in comparison to tree layer. There was no effect of seasonality on native coccinellid diversity in deciduous woodland, however native coccinellid diversity in coniferous woodlands was higher during the summer (t = -2.23, p = 0.02). The count part of the ZINB model revealed that native

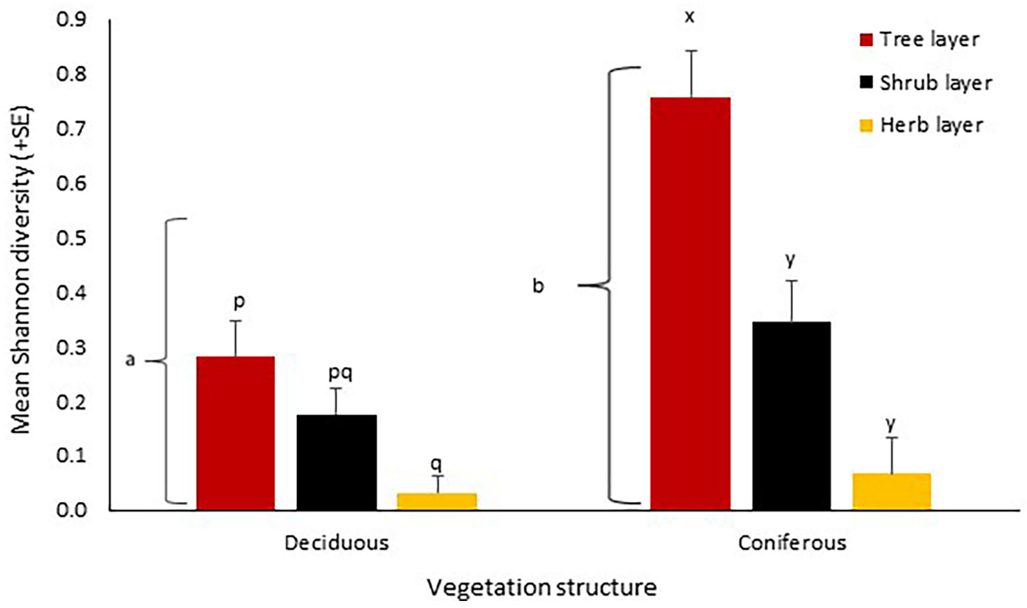

FIGURE 3
Mean Shannon diversity (+SE) of native coccinellid species at deciduous and coniferous sites and at different vegetation layers across all sites in Cambridgeshire and Suffolk. Consecutive letters indicate where significant differences occur. Letters on brackets represent differences between deciduous and coniferous sites collectively.

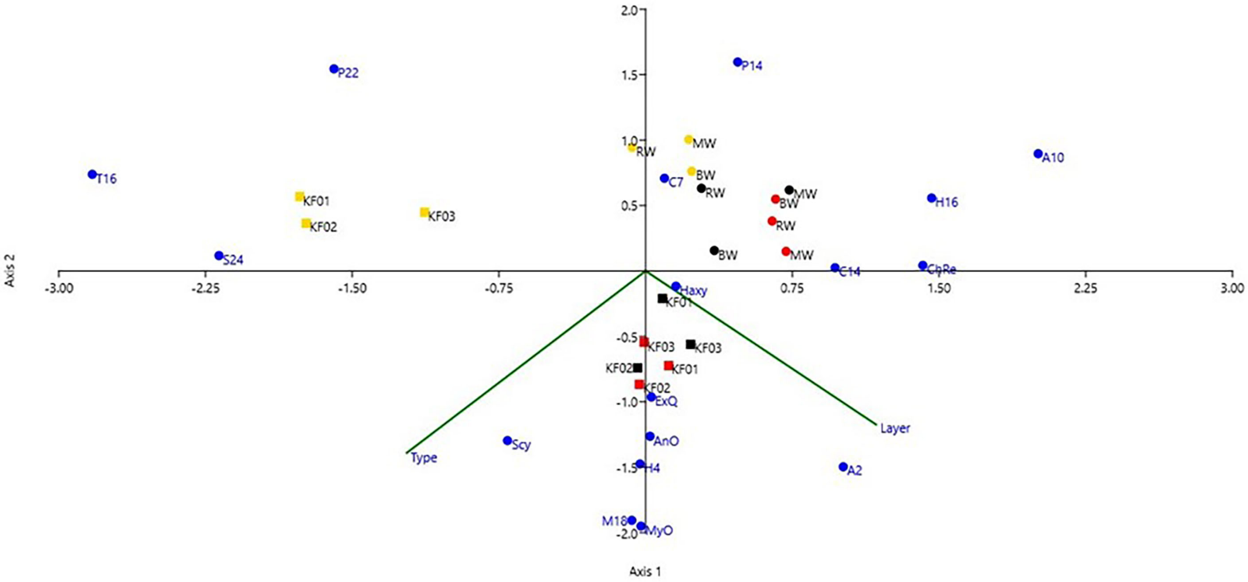

FIGURE 4
Species-conditional CCA triplot based on a canonical correspondence analysis of the coccinellid and environmental data recorded at rural sites in Cambridgeshire and Suffolk. Environmental vectors are amplified by a factor of two. Axis 1 explained 99.3% of the variation in the taxon-environmental structure and axis 2 explained 0.7% of the variation (Eigenvalues were 0.3505 and 0.0024 respectively); Type = coniferous sites (KF01, KF02, KF03 = Kings Forest site 1, 2 and 3) and deciduous sites (BW = Brampton Wood, MW = Monk's Wood, RW = Raveley Wood); Layer = tree, shrub and herb layer (coloured in red, black and gold respectively); coniferous sites are indicated by filled squares, deciduous sites by filled dots and coccinellid species by blue dots; coccinellid species = (A2, *Adalia bipunctata*; A10, *Adalia decempunctata*; AnO, *Anatis ocellata*; C7, *Coccinella septempunctata*; C14, *Calvia quattuordecimguttata*; ChRe, *Chilocorus renipustulatus*; ExQ, *Exochomus quadripustulatus*; H4, *Harmonia quadripunctata*; H16, *Halyzia sedecimguttata*; Hax, *Harmonia axyridis*; M18, *Myrrha octodecimguttata*; MyO, *Myzia oblongoguttata*; P14, *Propylea quatuordecimpunctata*; P22, *Psyllobora vigintiduopunctata*; S24, *Subcoccinella vigintiquatuorpunctata*; Scy, *Scymnus suturalis*; T16, *Tytthaspis sedecimpunctata*).

coccinellid diversity did not affect *H. axyridis* abundance, however, the binary model indicated that the probability of recording *H. axyridis* was significantly lower when native coccinellid diversity was higher ($z = -2.37$, $p = 0.01$). As expected, native coccinellid abundance was higher when native coccinellid diversity was higher ($z = 5.6$, $p < 0.001$).

Coccinellid assemblage

The coccinellid assemblage is represented by an ordination plot (Figure 4) which is interpreted below by starting with the environmental variables, Type and Layer. Focusing firstly on the 'Type' axis, there is a clear difference in the coccinellid communities that are associated with coniferous only and deciduous only sites. Some species are positively associated with coniferous (*M. oblongoguttata, M. octodecimguttata, Scymnus suturalis., H. quadripunctata*) and deciduous (*Halyzia sedecimguttata, A. decempunctata, P. quattuordecimpunctata*) sites while other species are more generalist and are associated with both sites in varying abundances (*H. axyridis, E. quadripustulatus, C. septempunctata*) (Figure 4). The 'Layer' axis also reveals that certain species are associated with particular vegetation layers and others are quite generalist in their habitat preferences. Habitat generalist species appear to aggregate along the centre of the 'Layer' axis (*H. axyridis, E. quadripustulatus, Scymnus suturalis, P. quattuordecimpunctata*) while the herb layer has a distinct coccinellid assemblage (*P. vigintiduopunctata, S. vigintiquattuorpunctata, T. sedecimpunctata*) (Figure 4).

Several species show a preference for the tree layer over the shrub layer in both coniferous (*A. bipunctata, M. oblongoguttata, M. octodecimguttata, H. quadripunctata, Anatis ocellata*) and deciduous sites (*Chilocorus renipustulatus, Calvia quattuordecimguttata, H. sedecimguttata, A. decempunctata*) (Figure 4). The herb layer at coniferous sites is distinct from the other coniferous vegetation layers and as expected is more similar to the deciduous herb layer (Figure 4). There was no difference in species diversity between the shrub and tree layer at deciduous sites and from the CCA plot (Figure 4) it becomes apparent that coccinellids use both vegetation structures with little variation between them, particularly when comparing the herb layer. For example, *C. septempunctata* (C7) is associated with both the herb and shrub layer, but with a greater abundance associated with the herb layer and a lower abundance associated with the tree layer. The tree and shrub layer at coniferous sites host similar coccinellid assemblages to

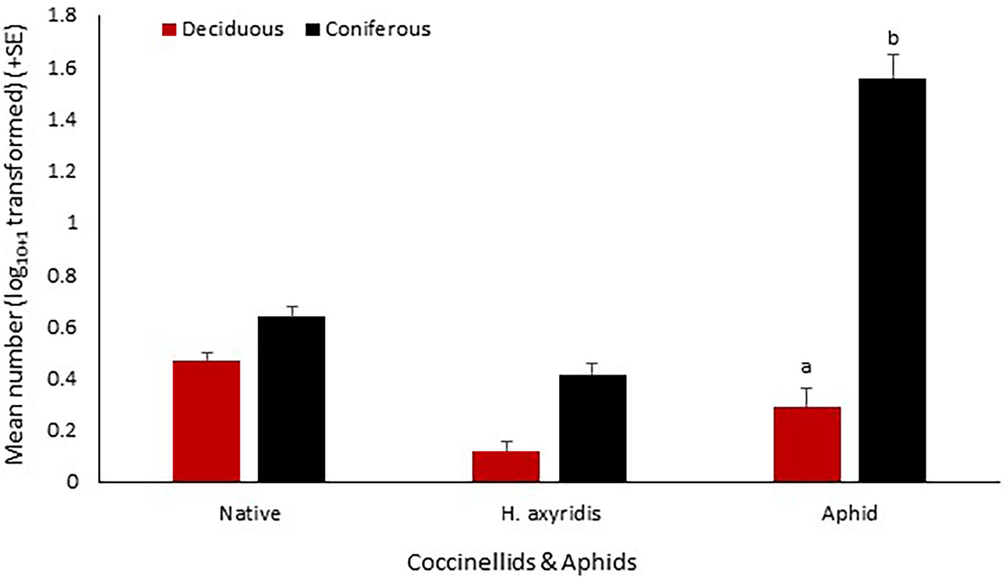

FIGURE 5
Mean number (log$_{10+1}$ transformed) (+SE) per site visit of *H. axyridis* and native coccinellids recorded in relation to records of Aphids in East Anglia. Native = native coccinellids recorded. Consecutive letters indicate where significant differences occur.

each other. For example, *E. quadripustulatus* (ExQ) was associated across all coniferous sites for both the tree and shrub layer yet has a greater association with the tree layer. Two coccinellid species (*T. sedecimpunctata* & *S. vigintiquattuorpunctata*) dominated the herb layer at coniferous sites that were not associated with any other site type or vegetation layer.

Harmonia axyridis appears as a generalist in the ordination diagram, being situated close to the origin and almost halfway on both variable axes. This species, however, was more positively associated with coniferous sites and with the shrub layer (KF01, KF03, KF02 & BW), while *H. axyridis* was negatively associated with the herb layer at both site types. Associations with certain native coccinellid species were evident, however these species were not as abundant as *H. axyridis*. Both *E. quadripustulatus* (ExQ) and *A. bipunctata* (A2) have a similar association with coniferous sites as *H. axyridis*, however *A. bipunctata* is positively associated with the tree layer, unlike *E. quadripustulatus* which seemed to utilise both the tree and shrub layer (Figure 4).

TABLE 3 Significance of Spearman correlations for coccinellid abundance with aphids recorded from trees at different site types.

Associated Insects	*Harmonia axyridis*	Native coccinellids
Aphids		
Deciduous Woodland	0.086	**-0.35**
Coniferous Woodland	**0.26**	-0.13
Deciduous Grassland	n/a	**-0.48**
Coniferous Grassland	n/a	0.10

Significant r^2 values are shown in bold.

Aphids

Across all site types (deciduous, coniferous) and vegetation structures (tree, shrub and herb layers) a total of 10,683 aphids (prey) were recorded. Woodland type had an effect on aphid abundance with significantly lower abundance at deciduous sites (z = -3.34, p = 0.0008; Figure 5). Vegetation structure had no effect on aphid abundance at either deciduous or coniferous woodland sites. *Harmonia axyridis* abundance was positively associated with aphid abundance at coniferous-only woodland while native coccinellid abundance was negatively associated with aphid abundance at deciduous-only woodland (Table 3). There was a negative association between aphid and native coccinellid abundance in deciduous grassland (Table 3).

Discussion

In this study, native coccinellids were more abundant than *H. axyridis* in both coniferous and deciduous woodland. This contrasts with studies on coccinellid assemblages in urban habitats which are dominated by *H. axyridis* (Brown et al., 2011a; Viglášová et al., 2017). When comparing deciduous and coniferous woodland habitats, the coniferous sites hosted a higher number of native coccinellids than did deciduous sites. Previous studies have suggested that coniferous habitat may act as a barrier to the continuing establishment of *H. axyridis*, thereby providing a refuge for native coccinellids from *H. axyridis* (Brown et al., 2011a; Purse et al., 2014), but our results do not support this. It is possible that *H. axyridis* has adapted to conditions found at coniferous sites in the UK and can overwinter successfully within them. *Harmonia axyridis* is highly phenotypically plastic and can adapt to available habitats, prey efficiently (Majerus et al., 2006) and climatic extremes (Sloggett and Majerus, 2000b).

When investigating overwintering coccinellid assemblages, Holecová et al. (2018) found that even though *H. axyridis* was one of the most abundant species on Scots Pine, the majority of the time, either *E. quadripustulatus* or *C. septempunctata* made up a larger proportion of overwintering coccinellids, being similar to that observed in this study. Coniferous woodlands experience less extreme temperature variation than deciduous woodlands (Ferrez et al., 2011) and greater overwintering success as a result could explain the increased abundance of native coccinellids at these sites. Additionally, considering the preference *H. axyridis* has for more sheltered overwintering sites, it is possible that the large area of coniferous plantation in this study provided sufficient shelter to enable this species to overwinter successfully. With a shortage of knowledge on coccinellid assemblages at overwintering sites (Pendleton and Pendleton, 1997-2019; Hodek, 2012; Holecová et al., 2018) investigating the overwintering coccinellid assemblages in coniferous woodland would provide further knowledge of coccinellid behaviour and importantly how climate change may influence coccinellid assemblages in the future. Another feature of the coccinellid life cycle that may be impacted by climate change is diapause. As the onset of diapause varies among coccinellid species (Hodek, 2012) the coccinellid community can vary. Studies on coccinellid diapause tend to focus on one of a small number of species and very little is known in relation to how diapause may impact coccinellid assemblages. Exploring the influence of diapause would lead to a greater understanding of the coccinellid community.

There was a distinct difference in the coccinellid community in relation to vegetation structure (tree, shrub and herb layer) between coniferous and deciduous sites. Within these individual site types, vegetation structure affected both the abundance and distribution of different species. The tree layer in both deciduous and coniferous woodland supported the greatest coccinellid diversity and in both cases differed to the herb layer. At urban sites, Vigláŝová et al. (2017) found that coccinellid diversity also differed across the different vegetation types that were surveyed, with higher species diversity in trees in comparison to nettle stands. The herb layer in this current study also hosted a different coccinellid community to that of the tree and shrub layer, likely due to the very different food sources available in the herb layer (mildew, plant material, different aphids). Similar findings were reported by Vigláŝová et al. (2017) for the coccinellid species observed on nettle stands. The differences reported in this current research relate to both site types with a unique coccinellid assemblage at both coniferous and deciduous sites. Grassland specialists dominated the herb layer at coniferous sites (e.g. *T. sedecimpunctata* & *S. vigintiquattuorpunctata*), while generalist coccinellids, such *C. septempunctata* dominated at deciduous sites. Interestingly, Vigláŝová et al. (2017) reported seasonal differences in how *C. septempunctata* used different vegetation structures, with greater numbers in nettles in the summer, and higher abundance on trees later in the year. No such seasonal effect was evident within our study, however, *C. septempunctata* did make use of the different vegetation layers as previously illustrated.

The abundance of *H. axyridis*, when present, was not influenced by native coccinellid diversity, however, when native coccinellid diversity was higher, the probability of occurrence of *H. axyridis* was lower. In an agricultural habitat, Grez et al. (2021) reported native coccinellid diversity to be negatively associated with non-native coccinellid abundance. It is widely documented that *H. axyridis* is often the most abundant coccinellid at urban sites, however this was not the case for rural woodland and grassland habitats in this study. *Adalia bipunctata* is reported to have high niche overlap with *H. axyridis* (Sloggett, 2008), however, the extent of co-occurrence between *H. axyridis* and *A. bipunctata* is likely to vary between habitats (urban/rural, tree/grass). Recently, Gardiner et al. (2021) reported native coccinellid diversity to be positively associated with forested habitat, while being negatively associated with urbanised habitats.

A negative relationship was observed between native coccinellid and aphid abundance at deciduous sites but at coniferous sites *H. axyridis* and aphid abundance were positively correlated. The majority of coccinellids recorded at deciduous sites were *C. septempunctata*, which is a species that is known to tolerate areas with low aphid density (Honěk, 1985). The third and fourth most frequently observed coccinellids at deciduous sites were *P. quattuordecimpunctata* and *A. decempunctata*, both of which are also tolerant of low aphid abundance (Honěk, 1985). The relationship between coccinellid abundance and that of aphids, however, is not an easy one to tease apart, in part due to the dynamic nature of both aphid and coccinellid populations. Vandereycken et al. (2013) reported a positive relationship between aphids and coccinellids in a range of crop habitats. Conversely, when investigating coccinellids in urban areas, Vigláŝová et al. (2017) found the relationship between common coccinellid species and aphid abundance to be non-linear with coccinellid abundance increasing with that of aphids, however when aphid abundance became very high, coccinellid abundance decreased. Furthermore, Brown et al. (2011a) and Brown and Roy (2018) did not find any correlation between *H. axyridis* or aphidophagous coccinellids and aphid abundance. A network ecology approach may reveal more about the complex relationship between coccinellids and aphids.

Conclusion and future work

The decline of native coccinellids is not solely a consequence of the arrival of *H. axyridis* and indeed some studies show that native species were in decline prior to its arrival (e.g. *A. bipunctata* & *C. quinquepunctata* in Czech Republic, Honěk et al., 2016). Climate change, land use change, intensification of agricultural practices (Honěk et al., 2016) and increased anthropogenic disturbance (Brown and Roy, 2018) may all have contributed to the decline of these species alongside the arrival of *H. axyridis*. There are suggestions that the initial decline of native species will reverse and that the invasive alien and native populations may stabilise and co-exist (Hentley et al., 2016). Research by Honěk et al. (2016) illustrates just how important long-term population studies are in having baseline data prior to the establishment of an IAS but also in determining how native coccinellid abundance can fluctuate over several years. Long-term studies in a range of habitats are needed to reveal a more complete picture on native coccinellid communities and how they change in the presence of IAS and other drivers of environmental change. Other process may be influencing the differences we observed between coniferous and deciduous sites, perhaps connectivity or adjacent land use or a combination of the two. Including these aspects in future work would enhance our knowledge of coccinellid communities and contribute to informing conservation action.

Coccinellid communities are not often the sole focus of studies and information on their structure tends to come as an add-on to other works (Honěk, 2012). More research needs to be initiated to investigate the coccinellid community as a whole and not just focus on individual species. In this study *H. axyridis* was less abundant than native coccinellids as a group within both rural woodland and rural grassland. A distinct native coccinellid assemblage was present within all three vegetation layers (tree, shrub and herb layer) sampled. Moving this research further forward, it would be interesting to explore how the coccinellid community varies with seasonality.

With increasing pressures from multiple drivers acting at varying temporal and spatial scales (Bonebrake et al., 2019), it is important to continue research into the dynamics of native coccinellid communities to inform appropriate conservation action. There is increasing evidence that the composition of landscapes can determine community composition (Gardiner et al., 2009), indeed Grez et al., 2014b demonstrated the importance of heterogeneous landscapes for increasing coccinellid diversity and abundance within agricultural systems. Indeed, conserving insect diversity, particularly as the pressure from global environmental change increases, will depend on improving spatial and temporal heterogeneity, including maintaining unique habitats and ensuring functional connectivity, to create landscape mosaics (Samways et al., 2020) that benefits insects including coccinellids. We have demonstrated the importance of different woodland habitats in supporting diverse coccinellid communities. Considering the complex relationship between aphids and generalist coccinellids it is important to further understand the importance of coccinellid diversity, how the communities exist in different landscape contexts and their role in ecosystem functioning.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

RAF, HER, PMJB contributed to conception and design of the study. RAF carried out field work and performed statistical analysis. RAF wrote the first draft of the manuscript. All authors contributed to revision of the manuscript, read and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcosc.2022.759046/full#supplementary-material

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Enhancing Our Understanding of Ladybirds in Ireland – A Case Study of the Use of Citizen Science

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Citizen science is increasingly recognised as a useful approach to contribute to biodiversity awareness while generating data and creating meaningful interactions among citizens and professional scientists. In a number of countries, academic institutions have led the way in developing ladybird projects, incorporating citizen science, to increase distributional data on the species, as well as a greater understanding of ladybird ecology. The current research evaluates the role of citizen science campaigns in collecting biological distributional data in Ireland, using ladybirds as a case example, examining if citizen science records and the use of photographs can provide a valuable insight into our understanding of ladybird ecology. From April 2020 to June 2021, a citizen science programme engaged with the wider public, *via* social media networks to increase the number of ladybird records and assist in establishing national data baselines, since ladybird rcording is a relatively new phenomenon in Ireland. This case study finds that citizen science projects using social media can increase the number of records of ladybirds and provide useful information on species distribution patterns. There were a disproportionate number of records from areas of high human population, resulting in a bias towards urban centres. Photographs submitted by members of the public provided a limited amount of information on food plants but indicated that ladybirds are valued as a species of interest to the public. These images can also help to increase records of the less common species, especially as members of the public may not be able to easily distinguish between different species of ladybirds.

Keywords: citizen science, Coccinellidae, social media, distribution, urban

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INTRODUCTION

The term 'citizen science' (or 'community science') has increasingly been featured in academic research over the last two decades and involves the participation of the public by collecting and providing data (Kruger and Shannon, 2000; Haklay, 2013; Ambrose-Oji et al., 2014; McKinley et al., 2017; Roche et al., 2021). It is evident from scientific literature that citizen science has been used in the tracking of environmental trends to increase numbers of biological records and therefore increase our understanding of ecological trends (Donnelly et al., 2014; Davis et al., 2018; Roche et al., 2021).

The data that can be collected by scientists depends on a range of factors, such as available funds, sample size and well-designed protocols (Gardiner et al., 2012; (Donnelly et al., 2014; (Bonney et al., 2016; Kosmala et al., 2016). Nevertheless, when few resources are available for research that requires data on the geographical distribution of species, citizen science is considered to be invaluable (Matutini et al., 2021), however, it has also been noted that in some cases, such as public outreach, citizen science projects can require lead-in time to gain momentum and awareness (Ferster et al., 2017). Citizen science, however, is a relatively new approach to dealing with data collection and scientific research. Geldman et al. (2016) recognised that there are tradeoffs in citizen science and that there are also spatial bias factors to consider. However, a growing body of publications suggest that accurate data can be produced for a diverse range of projects, if designed with a clear set of goals and appropriate methodologies (Kosmala et al., 2016).

Social media is an increasingly popular way of petitioning for records, offering free and open platforms (Mazumdar et al., 2018). Social media can impact the number of records submitted (Robson et al., 2013) by reaching a wider audience. Oliveira et al. (2021) examined the use of social media in citizen science projects and Roche et al. (2021) included "*Irish Ladybirds*" (the name of the social media page for this research) as one of the 42 citizen science projects in Ireland with over 1000 followers. Oliveira et al. (2021) highlighted the time required to set out the social media timeframes, posts, and analysis on the social media platforms but concluded that such platforms can be highly beneficial to biodiversity projects (Fota Wildlife Park, 2022). Furthermore, technological advances in smartphones, with good image capturing abilities, GPS co-ordinates are revolutionising citizen science and now enable records to be quickly shared (Mazumdar et al., 2018).

A number of studies have used citizen science approaches in ladybird research. These have included projects that have extended from the Americas to Europe and Africa ((Brown et al., 2007; Losey et al., 2007: Stals and Prinsloo, 2007; Steenberg and Harding, 2009; (Grez et al., 2016, Hiller and Haelewaters, 2019; Werenkraut et al., 2020; Angelidou et al., 2022). Citizen science has contributed to our knowledge on ladybirds—including new species records (Losey et al., 2007) and the spread of the globally invasive ladybird *Harmonia axyridis* (Stals and Prinsloo, 2007; (Roy et al., 2016; Hiller and Haelewaters, 2019; Werenkraut et al., 2020).

Recording of ladybirds on a national scale is relatively new in Ireland, and numbers of records have been increasing, greatly assisted by the establishment of the National Biodiversity Data Centre in 2007 (Roche et al., 2021). Datasets can be compared, by a system of cross-referencing data, with other data sources, linking to local weather at the time or recording, habitat types and the experience of the recorder ((Terry et al., 2020). The use of photographs can be invaluable for assessing relative abundances of rarer, less frequently recorded species, but also may reflect information on habitat associations ((Newcomer et al., 2019). However, the collection of data from photographs can also be influenced by patterns of human behaviour. Boakes et al. (2016) concluded that volunteers were often attracted towards particular schemes where they could identify species easily and where little effort was required.

In 2020–2021, we ran a citizen science programme in Ireland to increase the number of ladybird records, review distributional information, and evaluate if the collection of photo-records is a useful tool to gather information on the habitat and species present in Ireland.

METHODS

The study was based upon promotions using two Facebook platforms: (*i*) *Fota Wildlife Park* (a highly interactive page of 157,000 likes) which covers wildlife species in the park and other projects that are managed by Fota Wildlife Park and (*ii*) the research project Facebook page *Irish Ladybirds* (5699 likes). The Irish Ladybird project was promoted between April 2020 to June 2021, and records collected by email. The Irish Ladybird social media Facebook page was regularly updated, at a minimum on a weekly basis, with regular posts and a photographic competition held between April and September 2020. Promotional posts were incorporated into the work of Fota Wildlife Park which comprised of single postings. Records were collected *via* two email accounts. Information requested from members of the public included: (a) location (postcode/address/geo-coordinates) and (b) photographs of the ladybirds that were observed. Urban locations were identified as towns and villages with a human population more than 1500 (Central Statistics Office, 2021a). The photographs were examined to identify ladybirds and to collect habitat information. Photographic information was classified using criteria in **Table 1**.

TABLE 1 | Criteria used for assessment of photographs of ladybirds submitted by members of the public.

Information on the habitat	
Code	*Description*
1	No information shown on the photo of habitat; photograph shows ladybird only
2	Some information; photograph may show some details of plant substrate which is enough to identify the plant
3	There are clear details of the ladybird's surrounding
4	The ladybird is on a person
5	Ladybird is on an artificial surface, e.g., tarmac
Identification of ladybirds in the photographs	
6	The ladybird can be identified
7	The image is blurred but some identifying features can be seen
8	There is no useful information, and no features of the ladybird can be seen

RESULTS

A total of 924 records were submitted with 56% collected *via* the Irish Ladybirds Facebook page and 44% from the Fota Wildlife Facebook page ((Conrad and Hilchey, 2011). A total of 100 posts were posted on both Facebook platforms and from 2 to 16 posts per month (mean = 6.6, standard deviation = 4.1) with no significant relationship between the number or records and posts during the study period (r = -0.06516, p > 0.05). The largest number of people (38,000) viewed the original Facebook post on April 16, 2020. Social media engagement was enhanced by promotional activities as online posters encouraged the public to submit records of ladybirds in gardens and other local places. These were uploaded to the Irish Ladybird Facebook page on March 3, 2021, and consequently reached 11,300 people with 69 records submitted. To further encourage involvement a photo competition on the Irish Ladybirds Facebook page ran from April to September 2020 and elicited a total of 171 records.

Figures 1, **2** show the number of records submitted from areas where human population was less than 1500 and above 1500, respectively. The largest number of citizen science records were submitted from urban areas (>1500 human population) and furthermore from centres of high human populations (Dublin City and Cork City urban areas. The number of ladybird records submitted was highest in urban areas (> 1500 human population) (**Figure 2**). Note that out of the 924, total records, 28 records did not provide locality detail for mapping. The majority (84%) of records were submitted by one person for one location (**Table 2**). As a result, 685 people submitted records but only 16% of these submitted more than one record (**Table 3**). Monthly data indicate that records were higher in April, May, June, and July 2020 and for March and April 2021. The lowest numbers of records were submitted during the winter months (**Figure 3**).

We developed criteria to classify photographs in terms of identification of species and substrate. The majority of photographs (88%) gave a clear image of the ladybird, allowing accurate species identification. Of all photographs, 20% showed the ladybird only, whereas 20% gave a reasonable view of the substrate and surroundings. Ladybirds were mostly recorded on plant substrates, including Poacea spp. (grasses), *Urtica* spp. (nettles), and dead wood. A total of 30% of plant substrates in the photographs could not be identified, due to lack of detail or to the fact that only the ladybird was visible with no detail of surroundings. There was a common trend for recorders to pick up ladybirds and photograph them on their hand or arm.

Eleven species of ladybird were recorded in the survey (**Table 4**) representing 45% of the Irish ladybird biota. The most recorded species was *Coccinella septempunctata* (n = 1264 individuals), followed by the invasive *Harmonia axyridis* (n = 122). Our work has contributed to the number of records of two poorly known species, *Calvia quatuordecimguttata* and

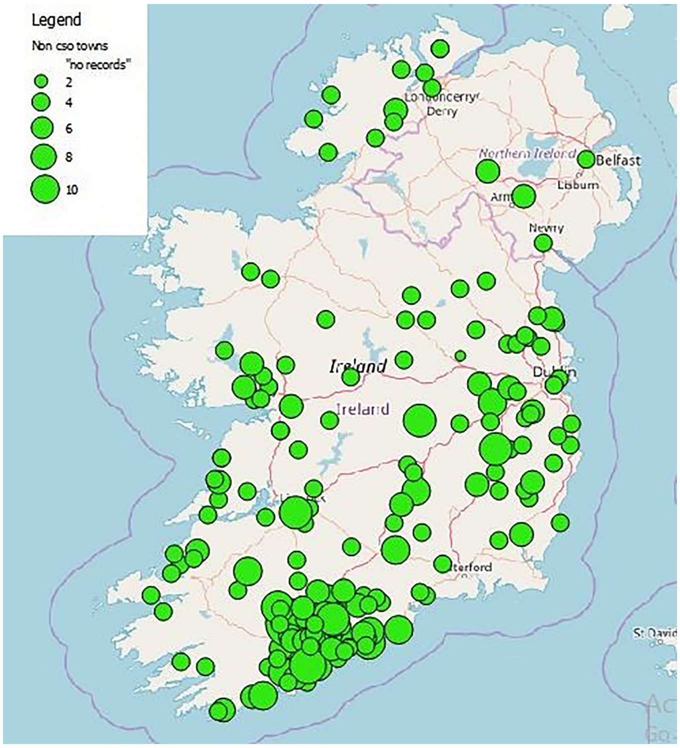

FIGURE 1 | Ladybird records submitted at locations with human population less than 1500 (Central Statistics Office, 2021a). Circle size reflects the number of records (total n=290).

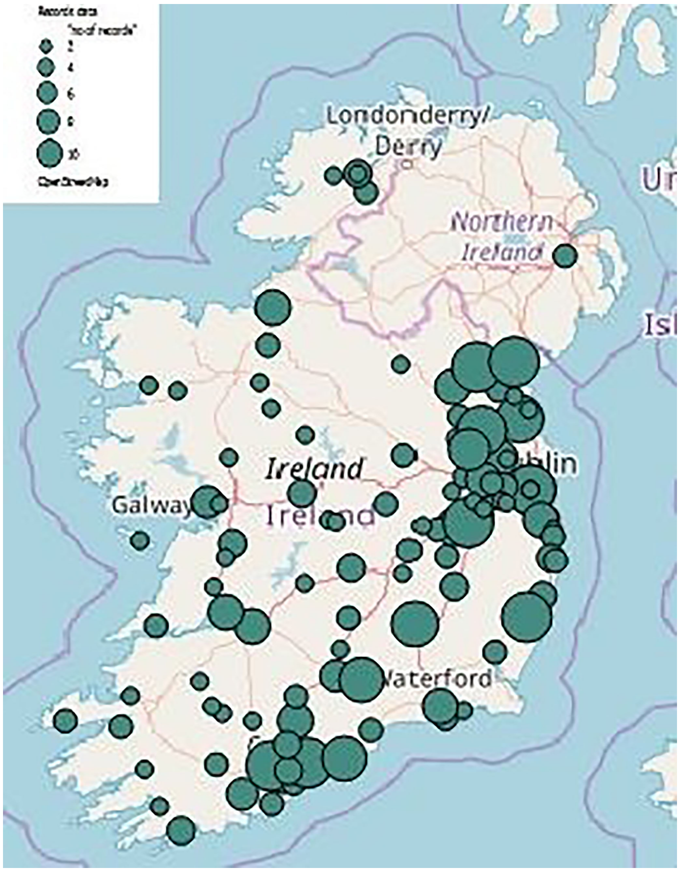

FIGURE 2 | Ladybird records submitted at locations with human population greater than 1500 (Central Statistics Office, 2021a). Circle size reflects the number of records (total n=606).

Chilocorus renipustulatus (**Figure 4**). Between April 2020 and June 2021, we recorded 36 records for these two species compared to 69 records reported by the national data base. Most of the species submitted to this research were generalist species that have a broad niche and can adapt to many environmental conditions. Specialist ladybirds have a narrower niche, e.g., *Chilocorus renipustulatus* is most often found on deciduous trees (Roy et al., 2011).

TABLE 2 | Number of ladybird records, no of people who submitted records and proportions of submissions (n = 685).

Number of records	Number of people who submitted records	Proportion of submissions (%)
1	576	84
2	67	10
3	23	3.2
4	9	1.6
5	3	0.4
6	3	0.4
7	2	0.2
15	1	0.1
47	1	0.1
Total	685	100

DISCUSSION

A considerable number of records were submitted as a result of a citizen science campaign in Ireland that ran for more than 15 months. There was a temporal trend in the submission of the records with a peak in late spring-early summer both years, with the emergence of ladybirds early in the year. Temporal trends have been observed by other citizen science research (Angelidou et al., 2022; (Knape et al., 2022) and attributed by (Kawakami et al., 2016) to predator and prey trends.

The pattern of human settlement across the country is reflected in the geographic distribution of records, with the majority of records submitted from small areas with large human populations. Most records were submitted from the two largest cities in Ireland, Cork, and Dublin. Nearly two-thirds of the Irish population live in urban areas (Central Statistics Office, 2021b; World Population Review, 2021) and

TABLE 3 | Locations recorded by an individual person, frequency, and percentage of frequency (n = 685).

Locations recorded by an individual person	Frequency	% Frequency
1	648	94
2	28	5
3	6	0.7
4	1	0.1
5	1	0.1
10	1	0.1
Total	**685**	**100**

the trend towards urbanisation of land in Ireland is among the highest in Europe, yet the proportion of urban to rural land cover remains at just below 2% (Ahrens and Lyons, 2019). The current finding that most records came from areas of high human populations is reflected within citizen science research (Ward, 2014; Geldman et al., 2016; Holden, 2017). Other research where citizen science has been used for ladybirds has revealed similar observations; records are higher in densely populated urban areas (Steenberg and Harding, 2009; Brown et al., 2018; Werenkraut et al., 2020)

Rocha et al. (2018) studied the relationship between aphids and ladybirds in urban gardens and concluded that increases in urban land cover do not favour ladybirds as urban garden habitats. There continues to be large gaps in data relating to our knowledge of the distribution and habitats of many species, and increased numbers of records in highly populated urban areas will give information on the distribution of only a small number of species (Howe et al., 2016).

Surveys on rural habitats are not common. Distribution maps created from other larger citizen science surveys, such as the UK Ladybird Survey with currently over 48,510 records (Brown et al., 2018). Recording of ladybirds in Ireland is a relatively recent activity and, while numbers have increased since the launch of the National Biodiversity Data Centre in 2007, the current survey has shown there are some species of ladybirds that are still under recorded. These include *Aphidecta obliterata*, *Chilocorus renipustulatus*, *Coccinella hieroglyphica*, and *Myrrha octodecimguttata* that are specialist species, in addition to being smaller in size and less well recognised as ladybirds by the public.

In this study, an increased number of records provided new information on the distribution and abundance of some ladybird species. *Coccinella septempunctata* was by far the most reported, followed by *Harmonia axyridis* and *Psyllobora vigintiduopunctata*. Species such as *Chilocorus renipustulatus* have been found to be more common in Ireland than anticipated. The first recorded sighting of this species was in Cork in 2011 (Weyman et al., 2019a), and records from the current citizen science campaign have shown its distribution to extend further out into the northern, southern, eastern, and western parts of County Cork. Eleven species of ladybird were recorded during the survey, representing two thirds of the total number of species recorded in Ireland (**Table 4**) (National Biodiversity Data Centre, *pers. comm.*). New knowledge relating to ladybirds can indeed be gained from citizen science data (Donnelly et al., 2014; Boakes et al., 2016; Hiller and Haelewaters, 2019; Roche et al., 2021). Although this survey produced a large number of records, the results highlight further gaps in ladybird distribution in Ireland that need to be addressed. The research identified not only spatial biases in a country with a widely spread rural population, but also that participation may reflect the motivation of the population to submit records. Ladybirds, being tiny can often be difficult to observe and this has been suggested to account for higher records of the larger more colorful ladybird species (Callaghan et al., 2019).

The first Irish record for *Harmonia axyridis* was made in 2007 in Antrim (Murchie et al., 2008). This invasive alien species is now established in Cork, Dublin, and Wexford (O'Sullivan, 2015; Weyman et al., 2019b). The rate of spread of *Harmonia axyridis*

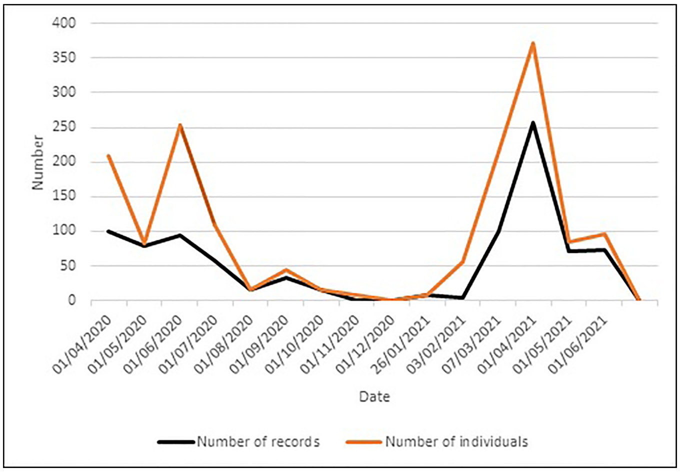

FIGURE 3 | Number of records (n = 924) and number of individual ladybirds (n = 1591) recorded between April 2020 and June 2021 during our citizen science campaign in Ireland.

TABLE 4 | Number of species and records of ladybirds recorded between April 2020 to June 2021 during our citizen science campaign in Ireland.

Species	Number of records	Number of individuals
Adalia bipunctata	34	43
Adalia decempunctata	24	35
Anatis ocellata	5	6
Calvia quatturdecimguttata	12	23
Chilocorus bipustulatus	1	2
Chilocorus renipustulatus	11	11
Coccinella septempunctata	708	1264
Harmonia axyridis	26	122
Myrrha octodecimguttata	48	50
Propylea quattuordecimpunctata	32	34
Psyllobora vigintiduopunctata	1	1
Total	**924**	**1591**

is lower in Ireland and other countries of northern Europe ((Roy et al., 2016), a pattern that could be explained by lower populations and climatic conditions (Roy et al., 2016). Despite a broad adaptability of *H. axyridis*, here are limitation as to where it can invade (e.g., Soares et al., 2017; Biranvand et al., 2019; Hiller and Haelewaters, 2019).

Social media is an invaluable tool for promoting awareness of topics and dissemination of information and can influence the number and quality of records submitted (Kosmala et al., 2016; Ellwood et al., 2017; Oliveira et al., 2021). Social media platforms are now commonly used for citizen science. Continuous engagement and eye-catching, colorful articles were found to increase the reach and interest from the Facebook posts on the ladybird project pages, a trend also noted by Lukyanenko (2019). Social media can be effective in targeting audiences (Robson et al., 2013) if they have a clear message. The current research found that asking for help to build up knowledge towards the protection of ladybirds resulted in considerable investment from the public. This could also be attributed to the likeability of ladybirds in part (Soares et al., 2022). It is unlikely that as many photographs would have been submitted if the taxonomic subject of the citizen science campaign was less appealing (Callaghan et al., 2019).

It was interesting to note that many of the records (n = 104) included a photo of a ladybird on the arm or hand of a person or child. This familiarity with ladybirds is reflective of the benign attitude that people have towards them. The connectivity between the natural world and humans has been frequently highlighted and ((Jaun-Holderegger et al., 2021) attributes this to experience and teaching of children by family members. Humans, especially children, clearly identify with ladybirds. There is a fascination with ladybirds due to their colour, importance in the garden for predating on plant pests and that the ladybird is associated with good luck in many myths and legends (Roy et al., 2013; (Majerus et al., 2017). There is undoubtedly more to be discovered about why people like ladybirds, and to explain why they are recognised, as a charismatic and popular species (Roy et al., 2013; Majerus, 2017; Soares et al., 2022).

Photographs are perceived as an invaluable analytical tool for collecting data (Catlin-Groves, 2012), and can be important for identifying species for research on estimating distribution (Suzuki-Ohno et al., 2027). Photographs from the 924 records were assessed for their ability to identify ladybirds and a classification system was used to establish their usefulness. Photos were found to

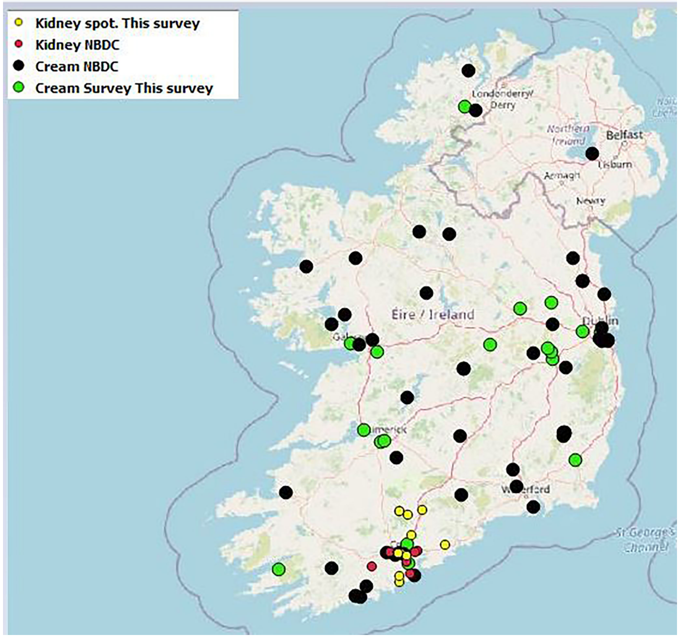

FIGURE 4 | Distribution of *Chilocorus renipustulatus* and *Calvia quatuordecimguttata* between April 2020 and June 2021.

be sometimes valuable in identifying ladybirds but did not always reflect the background characters required to identify the habitat or species of plant on which the ladybird was resting. Information on the immediate surrounding may be useful, e.g., wildflower areas, artificial substrates, et cetera. However, photographs submitted by the public were more often than not of little use in providing accurate substrate or habitat information. Citizen science has emerged as a useful tool for collecting data that are not otherwise recorded (Conrad et al., 2011). Verification of records using photographs is an important essential part of citizen science (Silvertown, 2009). Gardiner et al. (2012) suggested that the larger number of samples collected through direct citizen science may compensate for reduced accuracy depending on the types of data collected, but with clear identifiable photographs that can improve accuracy (Comont and Ashbrook, 2017). Citizen science-based research can also reveal sightings of invasive species which were previously unnoticed and provide valuable information where resources may be limited (Grez et al., 2016; Hiller and Haelewaters, 2019).

CONCLUSIONS

Citizen science is undoubtedly a useful tool in increasing our knowledge of ladybird distribution. Ladybirds are charismatic species and the public readily engages in providing information on these species. However, this study demonstrated that data provided by the public in response to a non-targeted social media campaign was predominantly urban in origin and centred on large cities resulting in a geographic bias in submitted records. While additional records extended the known distribution of some seldom recorded species, this citizen science campaign did not contribute records of ladybirds from across large areas of the country or from common habitats outside of cities. A substantial increase in effort is required to acquire the data that will fill these knowledge gaps.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of privacy concerns. Requests to access the datasets should be directed to the corresponding author, 112146854@umail.ucc.ie.

AUTHOR CONTRIBUTIONS

GW designed the survey, undertook data collection, analyzed data and drafted the manuscript. FB assisted in the drafting and revision of the manuscript. SM provided research facilities. All authors contributed to the article and approved the submitted version.

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