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Landscape structure and composition affect aphid biological control in alfalfa fields, but regional differences prevail

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With 2 figures and 1 table

Abstract: Understanding how landscape composition and configuration drive the dynamics of pests and their various natural enemies is crucial to enhance biological control services (biocontrol). Geographic variability in landscape effects prompts questions about whether this is due to genuine differences or methodological differences among studies, given that most studies are conducted in a single region. This study investigated how the ecological function of parasitoids and predators responds to landscape traits in three regions encompassing an agriculture intensification gradient. The effects of landscape structure and configuration differed among regions and natural enemies. Non-crop habitats increased the abundance of parasitoids only in two less intensified regions presumably due to insufficient amount of non-crop habitats in the intensified region (typically < 10%). Nevertheless, their biological control efficiency (BCE) was consistently increased by non-crop habitats across regions. Higher maize proportion increased predator abundance and their BCE in the two non-mountainous regions. The value of maize as a source habitat for predators may have been limited in the mountainous region due to the lower aphid abundances there. Edge density positively affected abundances of predators and parasitoids, but only positively increased the BCE of all natural enemies combined. Inconsistent regional responses to the same landscape variables suggest that a uniform integrated pest management strategy for alfalfa is impractical. However, increasing edge density should be a useful first step for supporting diverse natural enemy communities and their biocontrol potential.

Keywords: biocontrol efficiency; edge density; landscape trait; parasitoid; predator

1 Introduction

The presence of insect predators and parasitoids within agricultural landscapes is essential to suppress insect crop pests and conservation biological control contributes billions of dollars annually in reducing yield losses to pests (Foley et al. 2005; Losey & Vaughan 2006). Conservation biological control involves modifying the environment or existing agricultural practices to protect and enhance specific natural enemies or other organisms, thereby reducing pest abundance and crop damage (Eilenberg et al. 2001). Annual crops in particular, along with insufficient food resources such as nectar and pollen across an intensively cropped landscape in general, may not provide enough resources for natural enemies (Pfiffner & Luka 2000). The ephemeral nature and frequent disturbance of most agricultural landscapes pose challenges to pest suppression by natural enemies (Perović et al. 2021). To harness the full potential of biological control, it is imperative to understand the complex interplay between landscape factors and the dynamics of pests and their natural enemies (Perović et al. 2021).

The diversity and abundance of natural enemies are profoundly influenced by the structure of landscapes surrounding crop fields (Schellhorn et al. 2015; Zalucki et al. 2015; Nagy et al. 2020). This includes both landscape composition (e.g., the proportion of non-crop and crop habitat and habitat diversity) and landscape configuration (e.g., edge density, crop perimeter-to-area ratio, and field size) (Martin et al. 2019). Increased landscape complexity can substantially favor natural enemies, enhancing their access to resources

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and shelter, leading to increased predator populations and pest control (Hass et al. 2018; Sirami et al. 2019). However, depending on their biological traits (body size, feeding guilds, dispersal, diet breath etc.,) natural enemies may respond differently to landscape variables, a subject that has rarely been tested outside Europe and North America (Chaplin-Kramer et al. 2011; Martin et al. 2019).

Landscape effects on insect pests and their natural enemies can vary for multiple reasons. For example, different natural enemy guilds, comprising both parasitoids and predators, often possess unique resource requirements and species traits such as dispersal ability, leading to their diverse responses to landscape variables (González et al. 2021). Additionally, some insect pests benefit from non-crop habitats, challenging the generality of the relationship between non-crop habitats and biological control (Karp et al. 2018; Tscharntke et al. 2016). Consequently, it may be hard to generalize about the potential of non-crop habitats in supporting pests and/or natural enemy populations (Beillouin et al. 2021; Redlich et al. 2018). Landscape effects vary across regions due to differences in historical land use, landscape structure, land intensification, and farming systems. These factors play a key role in shaping the regional species pool (Aviron et al. 2005; Clough et al. 2005). While it is important to consider the increase in natural enemy populations, it is equally crucial to measure their regional-specific effectiveness in controlling pests when providing recommendations about landscape structure and composition that is optimal for reducing pest pressure.

Our study was conducted in an alfalfa cropping system. We assessed the responses of aphids and multiple natural enemies in alfalfa fields to the surrounding landscape composition and configuration in three regions spanning a wide geographic space and each characterized by different cropping structure. Alfalfa, *Medicago sativa* L., is the world's most valuable pluriannual forage crop. Aphids are a significant threat to alfalfa crops, causing production losses of 30% to 40% per year in the region (Shi et al. 2017). In northwestern China, it is a traditional component of crop rotations, and covers over 66% of the total area of forage, with approximately 0.4 million hectares grown annually (Fan et al. 2023). Moreover, alfalfa serves as a natural insectary plant and reservoir for natural enemies that regulate populations of alfalfa pests but also of pests of surrounding crops (Madeira et al. 2022). In our study, we tested the effects of landscape variables on two functional groups of natural enemies, i.e. predators and parasitoids. Predators are often considered as generalist enemies (Hurd 2008) whereas parasitoids are more likely to be host specialist (Gagic et al. 2016) and these and other traits are theoretically expected to respond to landscape variables differently (Corcos et al. 2017). We formulated three main hypotheses in our study. First, given that the population dynamics of aphids and natural enemies are strongly influenced by the landscape structure (Thies et al. 2005), we expected that higher proportions of non-crop habitat in the surrounding landscape would increase abundances of natural enemies. Second, given the different traits (e.g., diet breadth, dispersal ability) between predators and parasitoids (Alhadidi et al. 2019), the biocontrol efficiency (BCE) of these two functional groups may respond to landscape variables differently. In each sampled field we directly quantified BCE due to predators, parasitoids and their combined effect using exclusion experiments on sentinel plants and relate these measures to landscape attributes. Third, recognizing the ecological diversity inherent to different regions, regional characteristics should lead to context-dependent responses to landscape structures, impacting the abundance and biocontrol efficiency of predators and parasitoids.

2 Material and methods

2.1 Study areas

In light of the regional and variation in landscape attributes, we employed a stratified sampling approach in our study design. This study was conducted in three distinct regions within Ningxia Hui Autonomous Region in northwestern China in 2021. The three regions that were sampled exhibited distinct agro-ecosystems: 1) the intensified agriculture area (IAA), which was previously a desert area that had been transformed into farmland, had an average altitude of 1098 m and received 133 mm of annual precipitation; 2) the Agro-Pastoral belt (APB) was situated in an oasis area near the Yellow River, had an average altitude of 1302 m and received 251 mm of annual precipitation; and 3) the mountainous area (MA) was characterized by rugged terrain and had an average altitude of 1888 m and received 332 mm of annual precipitation. These regions spanned a distance of 396 km, from IAA to MA, and had varying vegetation patterns (Fig. S1). The agricultural landscapes in these regions consisted of a variety of crops, such as maize, orchards, vegetables, and alfalfa fields (Table S1). We selected a total of 24 farms as sampling sites, distributed as follows: 12 farms in MA, 6 farms in APB, and 6 farms in IAA. In each farm, we randomly selected three alfalfa fields as replicates giving a total of 72 fields. To prevent overlapping coverage, a minimum distance of 2.0 km was maintained between sampled farms. Landscape composition and configuration were characterized at radii of 0.5, 1.0, 1.5, and 2.0 km from the center of each focal alfalfa field.

2.2 Insect sampling

Sampling was conducted four times between July and early September, i.e., July 15 and July 29, 2021, and again from August 19 to September 3 2021, to coincide with the peak of the aphid population. Alfalfa aphids and their mummies (aphids parasitized by parasitoids with hardened and swollen aphid cuticle) were surveyed by direct counts on alfalfa stems. We inspected 500 alfalfa stems per field per sampling

date (5 random sampling points in the center of each field (i.e. at least 30m away from a field edge), with 100 stems inspected per point). To identify aphid species, we collected more than 100 specimens from each field and identified them in the laboratory. Two aphid species (Hemiptera: Aphididae), *Therioaphis trifolii* Buckton, i.e., spotted alfalfa aphid, and *Acyrthosiphon pisum* Harris, i.e., pea aphid, were found. At least 10 individual aphid mummies were collected on each sampling date and field, and reared in the laboratory for identification of parasitoids. The dominant primary parasitoid was *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae). Due to the inherent difficulty in directly counting adult parasitoid numbers, we used the numbers of mummies as an indirect indicator of parasitoid abundance.

Predators were sampled by sweep netting in the fields. Five parallel sampling transects, each 10 meters in length, were positioned perpendicular to the field edge to ensure comprehensive coverage of the field area. Total of 60 sweeps per field and sampling date were conducted using an 80 cm diameter net to capture and count ladybeetles and lacewings. The following species of predators were recorded: ladybeetles *Hippodamia variegata* Goeze, *Propylea japonica* Thunberg, *Harmonia axyridis* Pallas, and *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) and lacewings *Chrysoperla nipponensis* Okamoto and *Chrysopa phytochrome* Wesmael (Neuroptera: Chrysopidae).

2.3 Estimation of biological control efficiency (BCE) in alfalfa fields

Biological control efficiency (BCE) was assessed by comparing pest populations in cages excluding natural enemies with that in open fields. Two types of cages were set up using a modified approach based on Costamagna et al. (2013), and detailed information is provided in Fig. S2a. The full exclusion cage aimed to exclude all natural enemies, and the restriction cage aimed to exclude ladybeetles and lacewings but allowed parasitoids to enter. An open area with 0.25 m^2 containing approximately three alfalfa plants served as a control. Each cage enclosed an area of 0.25 m^2 containing approximately three alfalfa plants and was replicated four times in the center of each field. Each sampling farm had 12 cages in three fields (Fig. S2b), for a total of 288 cages across the three regions. These cages were positioned within each field at distance of 3 m between them, while maintaining a 10 m distance between adjacent replications.

Due to the regular cuttings of alfalfa, our strategy for BCE experiments was designed to coincide with the intercut, i.e., the periods between alfalfa cuttings. Field cages were established twice during the first and second intercut phases, with the second set of cages positioned slightly apart from the previous cage sites. To mitigate cage effects on the aphid population and dispersal, the experiment was limited to 14 days duration, in line with Gardiner et al. (2009). After this period, the cages were removed. Before initiating field experiments, all aphids and other arthropods were manually removed from caged plants and open field plots by using paintbrushes. Then, in each cage, 30 fourth instar nymphs or adult apterous *A. pisum* or *T. trifolii* (depending on the locally dominant aphid species) were manually released onto the highest central leaf of the plants using a paintbrush to simulate typical summer infestation levels. Between July 15 and July 29, 2021, and again from August 19 to September 3, 2021, we inspected cages weekly. The plants were then shaken manually to dislodge aphids onto a white plastic plate (450 mm × 600 mm) placed beneath them. Subsequently, apterous and adult aphids were counted and returned to the caged plants to reduce further disruption.

The BCE values were calculated from aphid data at 14 days after the cage installation. The BCEs of predators versus parasitoids can be evaluated by contrasting data sets from the exclusion cages with those from the open field (exclusion cage: total biocontrol efficiency; restriction cage: biocontrol efficiency from predators only). Furthermore, the BCE of parasitoids can be evaluated by comparing data from the restriction cages with data from the exclusion cages. To quantify the influence of natural enemies on aphid populations, we calculated the biological control efficiency (BCE), using the following equation (Gardiner et al. 2009):

$$BCE = \frac{1}{n}\sum_{P=1}^{4}\frac{(Ac - Ao)}{Ac}$$

Where Ac is the number of aphids in the cage treatment (natural enemies were absent or partially present), and Ao is the number of aphids with natural enemies present (aphid number values in the field corresponding to the particular cage type), *p* is the plot, and *n* is the number of replicates for a given site.

2.4 Landscape composition and configuration

We measured the surrounding landscape structure (composition and configuration) of each sampled alfalfa field at four spatial scales (radius 0.5, 1.0, 1.5, and 2.0 km). The types of land use were quantified with ArcGIS 10.4 software in combination with the remote sensing images (1 m resolution) of the Sentinel-2A/B satellite of the European Space Agency (ESA) from March to July 2021. Extensive ground verification was executed to validate the land use classifications. Five land use types were defined: alfalfa fields, maize fields (a dominant crop in the three regions, up to 70.77 %), other crops (including vegetable greenhouses, orchards, and wheat fields), non-crop habitat (comprising non-cultivated fields with annual grasses, abandoned fields, woodlands, and perennial weeds), and urban areas including dense residential roads, ponds, and industrial areas. Landscape composition was established by calculating the proportion of these five land-use types. Landscape compositional diversity was measured using Simpson's diversity index (SDI). Landscape configuration was characterized by edge density (ED) at the

landscape level. The calculation details for SDI and ED were based on McGarigal et al. (2002).

2.5 Statistical analyses

Abundances of aphids, parasitoids and predators were log-transformed ($\log_{10}(x + 1)$) to achieve normal distribution and homoscedasticity of residuals. The regional differences in the abundances of aphids, parasitoids and predators were tested by using one-way anova (ANOVA) and the pairwise differences were compared by using Tukey HSD post-hoc tests.

We used linear mixed effects models to analyze landscape effects on abundances of aphids and natural enemies, and on BCE of natural enemies. For each analysis, the response variable was selected from the following variables: abundance of aphids, of parasitoids (number of mummies), or of predators, as well as the BCE of parasitoids, of predators, or of combined natural enemies. The explanatory variables encompassed landscape composition: the proportion of alfalfa fields (Alfalfa), of maize fields (Maize), other crops (OC), of non-crop habitat (NCH) and of urban area (Urban); as well as landscape configuration: edge density (ED), and Simpson's diversity index (SDI). We set the observation days and fields (nested in regions) as random factors. These models were run separately for each spatial scale (0.5, 1.0, 1.5, and 2.0 km radius). All the explanatory variables were standardized by using scale function in R, which transforms these variables to a mean of zero and a standard deviation of one, aiding comparability and stability in linear regression analysis.

Explanatory variables strongly correlated (Pearson correlation $|r| > 0.7$) with other variables in the same model were excluded (Dormann et al. 2013). Notably, the proportion of maize and non-crop habitat was negatively correlated (Pearson $r = -0.69$, $p < 0.001$); so, they were tested in separate alternative models (set1: response variable ~ Alfalfa + Maize + OC + Urban + SDI + ED + random factors; set2: response variable ~ Alfalfa + NCH + OC + Urban + SDI + ED + random factors). Any variables displaying larger variance inflation factors (VIFs), VIF > 3, were omitted from models to avoid multicollinearity (Zuur et al. 2010). Based on Burnhan & Anderson (2002), all models with $\Delta AICc \leq 2$ were competitive models. If there were more than one best-fitting model, all competitive models were then subjected to a model-averaging procedure to determine parameter coefficients for the best final set of predictors. Following the identification of significant variables, we conducted a subsequent analysis to investigate the impact of the interaction between landscape factors and regions on the abundance and BCE of both predators and parasitoids.

To test the relative importance of direct and indirect paths (i.e., mediated through natural enemies) linking landscape structure and the BCE of natural enemies, we performed piecewise Structural Equation Models (pSEM) analyses. Landscape variables selected in the previous analyses were considered. We fitted a few models that included the landscape variables and links between natural enemies, pests, and BCE of natural enemies (see details in Table S4). The models were further simplified by removing non-significant paths if this improved model fit (Fisher's C with $p > 0.05$; $\Delta BIC \geq 2$; (Redlich et al. 2021)). The pSEM analyses were conducted separately at each scale (0.5, 1, 1.5, 2 km). We selected the scale with the highest Fisher's p-value to present in the main text, while the results for the other scales were presented in the supplementary file. The VIF values were calculated within the "car" package (Fox & Weisberg 2011), while LMMs analysis, model selection, and averaging utilized "lme4" (Bates et al. 2014), and "MuMIn" package (Mumin 2021). We used the "piecewise SEM" package (Lefcheck 2016) for SEM analyses. All analyses were performed in the software R version 4.2.3 (R Team 2023). Model validation was conducted by examining residual diagnostics for normality and heteroscedasticity, and by evaluating spatial and temporal autocorrelation using correlograms and Mantel tests, respectively.

3 Results

3.1 Aphid abundance

The abundance of aphids was greater in the plain regions APB and IAA compared to the mountainous region MA (Fig. S3). Across the four landscape scales, several landscape variables consistently influenced aphid abundance. Specifically, the proportion of alfalfa fields, and the proportion of maize showed a positive association with aphids, while ED and the proportion of OC exhibited a negative association with aphids (Table 1). The best prediction for aphid abundance was achieved at a scale of 0.5 km (model with lowest AICc scores; Tables 1 and S2).

3.2 Abundance of natural enemies

The abundance of parasitoids was significantly higher in the mountainous region MA in contrast to the plain regions APB and IAA (Fig. S3). Parasitoid abundance showed a consistent positive correlation with the proportion of non-crop habitat across the four scales in all regions (Table 1). Additionally, at the 0.5 km scale, there was a positive relationship with ED, but this trend was not observed at larger spatial scales. Parasitoid abundance decreased as the landscape diversity (SDI) increased but this was only significant at the 2.0 km scale (Tables 1, Table S2). The interactions between NCH and regions significantly affected parasitoid abundance for most models across the four scales, meaning that NCH affected parasitoid abundance differently across regions. Specifically, NCH did not affect parasitoid abundance in the IAA region, except for the marginal positive effect at the 1.5 km scale (Fig. 1a). However, NCH positively affected parasitoid abundance in the other two regions, except for no effect at the 0.5 km scale in the APB region.

Table 1. Summary of competitive models for the influence of landscape parameters on aphid abundance and on the abundance and control efficiency (BCE) of parasitoids, predators, and combined natural enemies in alfalfa fields at different scales. Best prediction scale (lowest AICc) was highlighted in bold. Model statistics are provided in Tables S2 and S3 for abundance and BCD models, respectively. BCE: Biological control efficiency; ED: Edge density; Maize: proportion of maize; OC: proportion of other crops; SDI: Simpson's diversity index; NCH: proportion of non-crop habitat. The symbol "−" indicates a negative correlation, while "+" indicates a positive correlation.

Response Variables	Radius (km)	ID	BCE	Abundance
Aphids	0.5	1		**Alfalfa − ED + Maize − OC + SDI**
		2		Alfalfa − ED + Maize + SDI
	1.0	1		Alfalfa − ED + SDI
		2		Alfalfa + SDI
	1.5	1		Alfalfa − ED + SDI
	2.0	1		Alfalfa
Parasitoids	0.5	1	NCH	NCH
		2		ED + NCH
	1.0	1	NCH	NCH
	1.5	1	NCH	NCH
	2.0	1	**NCH**	**NCH − SDI**
Predators	0.5	1	− NCH	− Alfalfa + ED + OC − SDI
	1.0	1	**ED − NCH**	Maize + NCH + OC − SDI
		2	ED − NCH − Urban	− Alfalfa + Maize + NCH − SDI
		3	− NCH − Urban	− Alfalfa + Maize + SDI
		4	− NCH	
	1.5	1	− NCH	**− Alfalfa + ED + Maize + NCH − SDI**
		2	− NCH − Urban	− Alfalfa + ED + Maize − SDI
		3		ED + Maize + NCH + OC − SDI
		4		− Alfalfa + ED + Maize + NCH + OC − SDI
	2.0	1	− NCH	− Alfalfa + Maize + NCH − SDI
		2	Maize + OC	Maize + NCH − SDI
		3		− Alfalfa + Maize + NCH + OC − SDI
Combined natural enemies	0.5	1	**ED**	
		2	− Urban	
		3		
	1.0	1	− Urban	
		2	ED	
		3		
		4	ED − Urban	
		5	ED + Maize + NCH + OC	
	1.5	1	Maize + NCH + OC	
		2	− Urban	
		3	NCH	
		4		
	2.0	1	Maize + NCH + OC	
		2	− Urban	
		3	Maize + NCH	
		4	NCH	

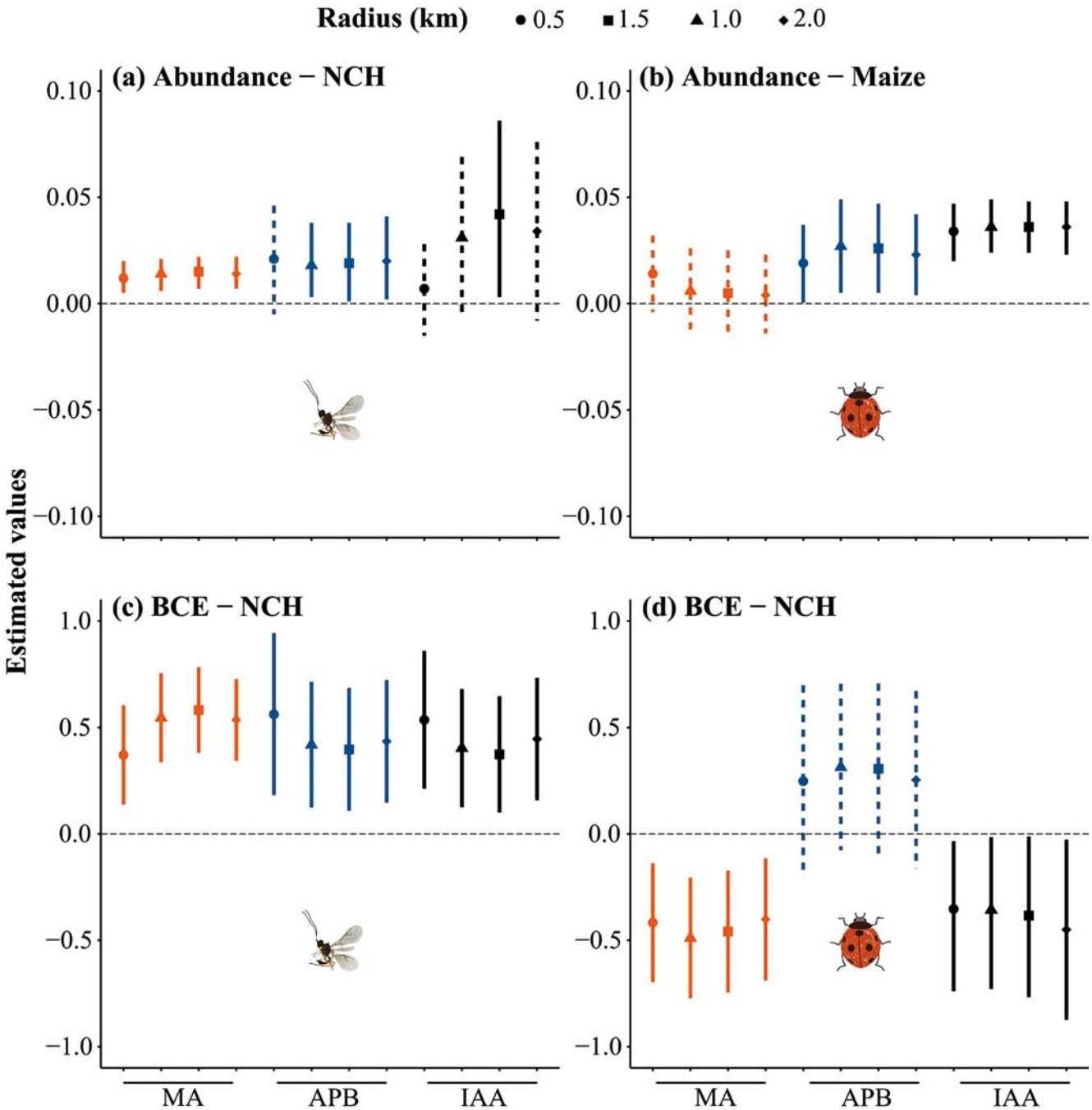

Fig. 1. Effects of the interaction between the proportion of maize, non-crop habitat (NCH) and region (MA = Mountainous Area, APB = Agro-Pastoral belt, IAA = Intensive Agriculture Area) on **(a)** the abundance of parasitoids, **(b)** the abundance of predators, **(c)** the BCE (biological control efficiency) of parasitoids, and **(d)** the BCE of predators across four spatial scales (shown by different dot patterns). Solid lines mean significant, dashed lines mean not significant.

The abundance of predators was greater in plain regions IAA and APB compared to the mountainous region MA. The abundance of predators was positively associated with ED, the proportion of maize, of non-crop habitat, and of other crops at most landscape scales (Table 1). Nevertheless, predator abundance decreased as the landscape diversity and alfalfa proportion increased at all scales (Table 1). The interactions between maize and regions (IAA and APB) were significant in models predicting predator abundance. Specifically, the proportion of maize in the landscape had no effect on predator abundance in the MA region (Fig. 1a), while it positively affected predator abundance in the other two regions across all four landscape scales.

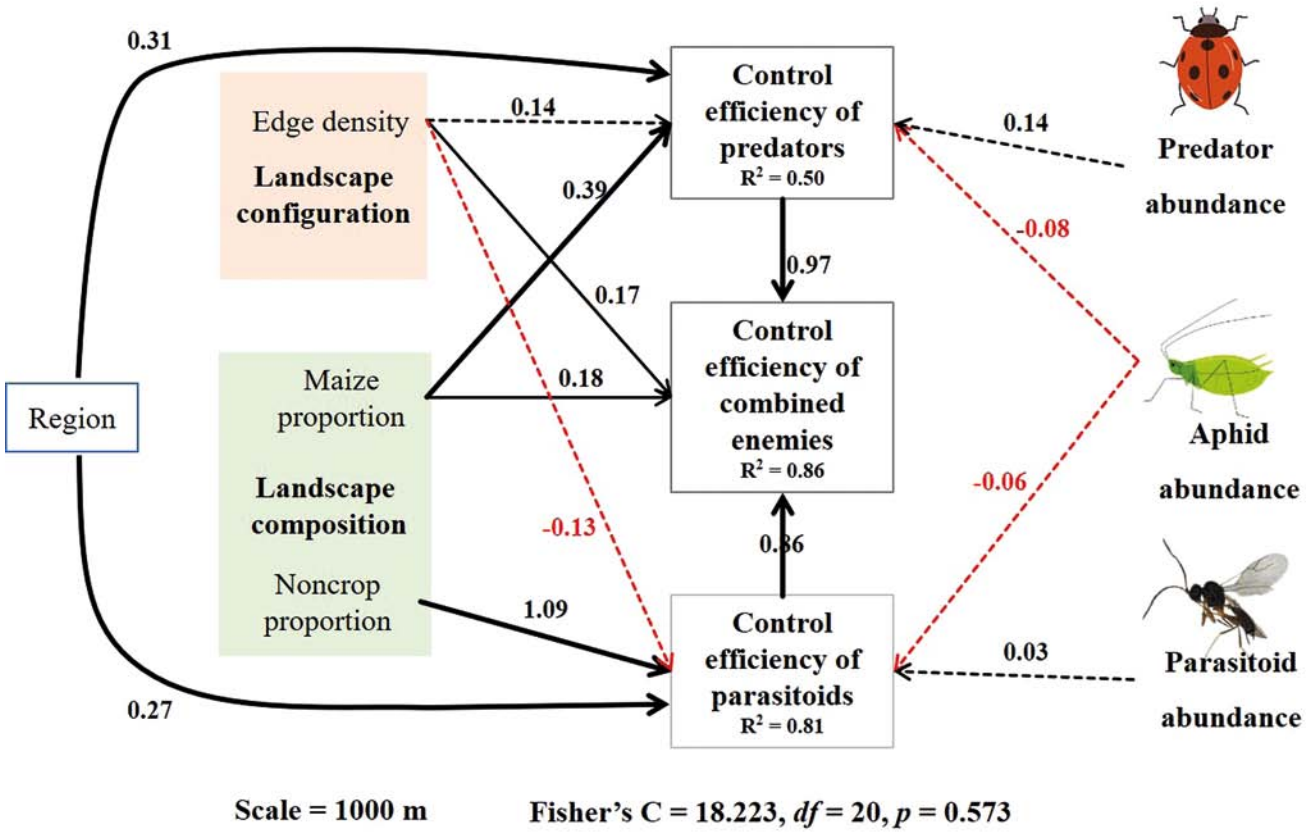

Fig. 2. Piecewise SEM on the multiple relationships between the abundance of aphids and of natural enemies, the biological control efficiency of natural enemies (response variables), and landscape predictors as well as region effect at 1 km scale (best prediction scale for piecewise SEM analysis). The standardized estimate for each line is shown next to each line. Black and red lines indicate positive and negative relationships, respectively. Plain and dashed lines show significant and non-significant relationships, respectively. R^2 values are shown for response variables.

3.3 Biological control efficiency of natural enemies

The biological control efficacy (BCE) of parasitoids was significantly higher in MA (60.6 %) than in APB (31.5 %) and IAA (29.7 %), whereas the BCE of predators was significantly higher in IAA (72 %) compared to MA (61 %) and APB (60.7 %) (Fig. S4). Overall, the BCE of combined natural enemies was significantly higher in MA (84.9 %) than in APB (75 %) and IAA (73.9 %). BCE of parasitoids was positively associated with non-crop habitat proportion at all scales (Table 1, Table S3). Conversely, the BCE of predators exhibited varying patterns, with positive correlations with ED at the two smallest scales (0.5 and 1.0 km) and with the proportion of maize and of other crops at the 2.0 km scale. However, there were negative correlations with the non-crop habitat proportion at all scales (Table 1). Regarding the region effect, BCE of parasitoids was positively and significantly affected by NCH across all regions, whereas maize proportion had a significantly negative effect on the BCE of predators in regions APB and IAA, but not in MA (Fig. 1).

BCE of combined natural enemies was positively associated with ED at two smaller scales (0.5 and 1.0 km) and with the proportion of maize, non-crop habitat, and other crops (Table S3). ED had a significantly positive effect on the BCE of combined natural enemies only in the APB region and across all spatial scales (Fig. S5).

3.4 Piecewise Structural Equation Models (pSEM)

The pSEMs revealed divergent responses in the BCEs of parasitoids versus predators (Fig. 2). NCH proportion positively influenced the BCE of parasitoids, while maize proportion positively affected the predators' BCE as well as the BCE of combined natural enemies. At the same time, the region effect was significant on both the BCEs of parasitoids and of predators (Table S4; Fig. 2). Neither abundance of parasitoids or predators was significantly associated with their BCEs. Overall, the significant relationships between landscape variables, region and natural enemies' BCEs were similar across the four spatial scales (Fig. 2; Fig. S6). While the significance of region consistently persisted in all cases, the influence of landscape variables occasionally failed to reach significance, suggesting varying levels of impact across the spatial scales examined.

4 Discussion

Our study revealed that landscape composition and configuration significantly affected parasitoids and predators' abundances and their associated biological control efficiencies (BCEs) in different ways with variation across regions and landscapes scales. Specifically, non-crop habitats supported higher parasitoid abundances (in the two less intensified regions) and higher parasitoid BCE (in all regions), but lower predator BCE. However, the abundance of predators and their BCE were positively correlated with the percentage of maize in the landscape in the non-mountainous, plain regions. Edge density (ED) positively affected the BCE of combined natural enemies. The landscape effects varied across regions and scales. Non-crop habitats were positively associated with parasitoid abundances in mountainous areas across all scales, although the association was less consistent in plain regions.

4.1 Landscape effects across functional groups

We found that non-crop habitats exerted a significant positive impact on parasitoids abundance, which in turn improved their biocontrol efficiency. Non-crop habitats likely provided essential resources and favorable conditions, such as flower nectar and alternative host, fostering the reproductive success and persistence of parasitoid populations (González et al. 2022).

We found maize cropland positively influenced predator abundance. Maize cropland, known for hosting a substantial quantity of cereal aphids, could act as a source habitat for ladybeetles, thereby augmenting biological control measures against aphid pests in adjacent crop fields (Ouyang et al. 2012). Ladybirds will tend to lay eggs where there are abundant aphids, as often observed in maize field, this could boost their population growth. Edge density also positively affected predators, which suggests that more field boundaries facilitate movement of predators between maize and alfalfa fields in the present study.

There was a positive association between non-crop habitats and predator abundance. However, the increased abundance of predators in response to non-crop habitats did not translate into the expected improvement in biocontrol efficiency. Surprisingly, we found a negative association between non-crop habitats and predator BCE. This negative relationship may be attributed to the association between non-crop habitats and maize, as indicated in our materials and methods section, where these two variables were found to be negatively correlated. Therefore, the negative association between non-crop habitats and predator BCE may not imply a causal relationship but could rather be attributed to the potential confounding effect of maize, which is known to benefit predator abundance and may consequently influence biocontrol efficiency.

Natural enemies responded differently to landscape composition. The responses exhibited by predators and parasitoids reveal intricate dynamics influenced by landscape variables and are likely due to their species characteristics, such as specialization and dispersal (González et al. 2021). Predators, typically generalists, exhibit frequent movement between crop and nearby habitats, thereby potentially experiencing influences from nearby habitats in crop fields – a phenomenon similar to mass effects (Urban 2006). Conversely, parasitoids, which specialize in targeting aphids, may benefit from the presence of non-crop habitats not only as a potential source of aphids but also for alternative resources such as nectar. This suggests a spillover effect where non-crop habitats contribute to the provision of essential resources for parasitoids, enhancing their fitness and potentially their biocontrol efficiency within crop fields.

4.2 Scale effects vs. regional effects

Our investigation highlights the need for a thorough examination of the scale at which landscape variables exert their influence on the intricate dynamics of aphid-natural enemy interactions. Specifically, edge density effects were observed at a smaller scale (< 1.5 km) within the landscape, suggesting that the effects of edge density are notably localized in our study. The best-fit models for predators were at a scale of 1 km, whereas parasitoids had optimal fit at a scale of 2 km. Literature supports the idea that generalist enemies respond consistently to landscape complexity across all scales measured, while specialist enemies exhibit a stronger response to complexity at smaller scales (Chaplin-Kramer et al. 2011). Our result is not consistent with this finding. One plausible explanation is that our studied scales were all relatively small (maximum 2 km) and needed to be larger to capture this effect.

The landscape effects on insect abundance varied across regions. For instance, maize cropland significantly and positively impacted predator abundance only in the plain regions (APB and IAA), where a higher proportion of maize cropland was observed. Moreover, aphid populations in APB and IAA were more abundant compared to the mountainous region (MA). Distinguishing between regional differences and landscape effects stemming from maize cropland variation poses challenges. Nevertheless, this observation reinforces the notion that maize fields serve as a significant food source for predators, contributing to the enhancement of their populations.

Our results showed that the BCE of parasitoids and the combined natural enemies in MA (parasitoids: 60.6 %, combined natural enemies: 84.9 %) were consistently higher than in the other two regions (APB and IAA). These findings suggest landscape context is crucial when designing an IPM strategy. The observed regional differences in the responses of pest aphids, their parasitoids, and predators suggest that a one-size-fits-all approach to integrated pest management in alfalfa may not be effective. Tailoring pest control strategies to the specific characteristics of each region we believe is crucial, such as increasing percentage of non-crops for

providing higher BCE of parasitoids in intensive agriculture area, while increasing cropland percentage and edge density for offering more predators and higher predator BCE in mountainous area.

Therefore, moving forward, we suggest researchers need to not only work at landscape scale but also across larger regional scales when addressing context-specific effects of landscape characteristics. In addition, measures of abundances of pests and natural enemies need to be complemented with experimental measures of their impacts, i.e. biocontrol efficiency, which is not always positively correlated with natural enemy abundances (Zalucki et al. 2015). These could be made more relevant if they included measures of yield benefits and insecticide use in commercial farms (Furlong & Zalucki 2010). This may lead to improved pest management strategies in agricultural landscapes, but also a better implementation of such strategies.

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Table S1–S4, Figure S1–S6