#### ORIGINAL ARTICLE



# Management practices influence biocontrol potential of generalist predators in maize cropping systems

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#### Abstract

Intensification of conventional agriculture is a leading cause of negative environmental impact, loss of biodiversity, and reduced delivery of ecosystem services in agroecosystems. Maize, due to its growth habits and cultivation management, provides a poor habitat for beneficial arthropods. Several strategies are available to make maize cropping systems more sustainable and to promote biodiversity at field level. The present study evaluates the effects of various maize cropping systems – precisely, maize continuous monoculture, maize multiple cropping, and three mixed cropping systems (maize-runner bean, maize-sorghum, and maize-flower strips) - on generalist predators and their biological control potential. Overall, we found that the reference system with maize continuous monoculture and conventional crop management had the lowest activity of generalist predators compared in particular to the low-input mixed cropping systems. Higher activity density and biocontrol potential were found in the systems that provided a dense and permanent vegetation cover of the ground (i.e., maize/sorghum, maize/flower strip). Although these effects were not consistent for all the parameters investigated and for every sampling date, we conclude that generalist predators can benefit from more conservative management practices in maize systems, thereby enhancing their biological pest control potential. Furthermore, spatial intercropping may represent a valid alternative to the conventional monocultural crop system to support the delivery of biodiversity-mediated ecosystem services towards a more sustainable system.

#### **KEYWORDS**

agroecosystems, beneficial arthropods, biocontrol agents, biological control, crop diversification, ecosystem services, flower strips, generalist predators, intercropping, low-input maize, maize mixed cropping, Rapid Ecosystem Function Assessment

# INTRODUCTION

Over the last decades, intensification of conventional agriculture has mainly aimed to increase productivity, leading to negative environmental impacts on soil, water, air, and biodiversity in agroecosystems (Tilman et al., 2002; Landis, 2017). Simplification of farming systems has caused an increasing decline of multifunctionality of agricultural landscapes (Rusch et al., 2016; Lichtenberg et al., 2017), thereby threatening the provisioning of ecosystem services and biodiversity (Tscharntke et al., 2012). Agricultural production is highly dependent on ecosystem services such as pest control and pollination amongst others (Power, 2010). Functional agrobiodiversity, through ecological processes and functions (e.g., predation, flower visits, mineralisation), allows the provision of regulating services (e.g., pest control, pollination, nutrient cycling), that benefit agricultural systems (Zhang et al., 2007). Therefore, implementing appropriate agricultural management practices in order to improve the productivity, but at the same time sustaining the delivery of ecosystem services and minimizing environmental

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costs, remains one of the main challenges of a modern and more sustainable agriculture (Power, 2010; Tscharntke et al., 2021), towards an ecological intensification of crop production (Kleijn et al., 2019).

In agricultural landscapes, habitat heterogeneity has the potential to affect biological pest control enhancing natural enemy density and reducing crop pest pressures (Thies & Tscharntke, 1999; Bianchi et al., 2006; Letourneau et al., 2011). In a meta-analysis, Letourneau et al. (2011) demonstrated that diverse agroecosystems show less pest damage, fewer herbivores, and more natural enemies than less diverse cropping systems. Spatial diversification of crop and non-crop habitats in farming systems can enhance natural regulation of insect pests (Hatt et al., 2018), mobilising predators and parasitoids which can exert a top-down control of insect herbivores (Gurr et al., 2003). A more diverse habitat can offer these natural enemies a shelter against adverse conditions, overwintering sites, floral resources, alternative prey, and hosts (Gurr et al., 2017). At field scale, it is generally acknowledged that increasing crop diversity and structural complexity may support higher abundance and diversity of natural enemies, thereby enhancing biocontrol services (Landis et al., 2000; Langellotto & Denno, 2004; Iverson et al., 2014). Crop diversification practices such as intercropping may therefore represent an option to increase biodiversity in agricultural ecosystems towards a higher biological control (Barbosa, 1998), as shown by studies carried out in various arable cropping systems (e.g., Wang et al., 2009; Zhou et al., 2013; Lopes et al., 2015; Puliga et al., 2022) as well as in vegetable systems (Juventia et al., 2021).

Conventional maize cropping systems are especially considered to have negative effects on biodiversity due to the growth habits of the crop and the large amount of external input necessary for its cultivation (Immerzeel et al., 2014; Norris et al., 2016). Soil preparation and weed control during the initial stage of the growing season result in a large amount of bare ground (Hall et al., 1992), which makes the maize field a poor habitat for above- and below-ground arthropods (Norris et al., 2016). Nevertheless, several maize cultivation practices are available to reduce negative environmental impact of this crop, including reduced tillage, mechanical weeding, cover cropping, undersowing, intercropping, etc. (Giuliano et al., 2016). Maize spatial intercropping is a common cultivation strategy but so far only widespread in several (sub-)tropical countries. In these countries, maize is mostly mixed with legumes, vegetables, and cereals (Batugal et al., 1990; Li et al., 2001; Lopez-Ridaura et al., 2021). Such spatial intercropping strategies have been drawing more attention also in European agricultural systems, and a study carried out in Germany (Fischer et al., 2020) showed that maize yield of a maizebean mixed cropping system is comparable to that of a sole maize crop. Also mixing maize with no crop elements such as flower plants has been demonstrated to

affect beneficial arthropods, attracting pollinators and increasing their density and diversity (Norris et al., 2018). It is known that generalist predators such as Carabidae (ground beetles) and Araneae (spiders) can play an important role in maize cropping systems contributing to the control of herbivore populations. Field experiments demonstrated that ground beetles and spiders are able to reduce populations of various pest groups in maize, in particular Cicadellidae and Thysanoptera, with also Aphididae being affected (Lang et al., 1999). Nevertheless, the effects of maize cultivation strategies on these beneficial organisms still remains little investigated.

In The Netherlands, where this study was conducted, silage maize represents one of the most important cultivated crops (around 195000 ha in 2020; Dutch Central Agency of Statistics, 2021). In order to make maize production systems more sustainable, alternative cropping strategies with focus on crop diversification in time and space are currently under investigation. Here, different maize cropping systems are compared. Conventional maize monoculture (M\_M) represents the reference system of the experiment and it is compared with four low-input maize cropping systems. In M\_M the soil is maintained bare during the fallow period in winter. In the multiple cropping system (M MC), a temporal variation of the rotation is added through the cultivation of a winter cover crop. Winter cover crops are implemented before the cash crop for their benefits, which include contributions to weed and invertebrate pest control. In particular in low-input cropping systems, cover crops may play a fundamental role offering additional ovipositional sites and refugia for natural enemies during winter time (Lundgren & Fergen, 2011a, 2011b). Furthermore, three maize mixed cropping systems are implemented. These treatments involve the cultivation of a winter cover crop and three different intercropping strategies: maize with sorghum (M S), maize with runner bean (M RB), and maize with flower strips (M FS).

In this study, applying the 'rapid ecosystem function assessment' (REFA) approach (Meyer et al., 2015), a set of ecosystem function proxies relevant for biological pest and weed control was measured. Using sentinel prey artificially placed in the field, which represents a useful and widely implemented method in agroecological studies (Lövei & Ferrante, 2017), it is possible to quantify the potential contribution of those predators to biological control. Furthermore, the activity density of the most important groups of generalist predators was also assessed.

We hypothesized that (1) the low-input systems would support higher activity density of epigeal predators compared to the conventional maize cropping system, thereby enhancing also their biocontrol potential. Furthermore, among the low-input cropping systems, we expected that (2) the treatments with increased ground cover and crop diversity would benefit activity of epigeal predators and enhance biocontrol potential.

# MATERIALS AND METHODS

# **Description of the field experiment**

This study was conducted in 2019 and 2020, at an ongoing field experiment of Wageningen University and Research near Lelystad, Flevoland, The Netherlands (52°31′47″N, 5°33′37″E). This long-term field trial was established in 2009 with the aim of studying sustainable silage maize crop management practices, with focus on soil tillage, weed control, and cover crop cultivation. The trial is divided into three blocks (three spatial repetitions), and each block has six experimental strips with 10 experimental plots. Plots are  $4.5 \times 12$  m, resulting in a size of the entire trial field of around 2 ha, including the buffer space between plots. Since spring 2018, one of the main objectives of this trial has been to test different crop diversification strategies in order to break monocultures of maize. Several treatments have been implemented, investigating both temporal and spatial diversification strategies of maize cropping systems. Five treatments were selected for our study: (1) maize continuous monoculture (M\_M, reference), (2) maize multiple cropping (M MC), (3) maize-runner bean mixed cropping (M RB), (4) maize-sorghum mixed cropping (M S), and (5) maize-flower strip mixed cropping (M FS) (Figures S1 and S2). All the treatments were implemented every year on the same plots. In the system M\_M, maize was cultivated continuously as sole crop, representing the reference treatment of the experiment. No winter catch crop was cultivated in this treatment. In the multiple cropping system (M MC), temporal diversification of the maize system was achieved growing a winter catch crop, i.e., a mix of rye, Secale cereale L., and winter pea, Pisum sativum L. In terms of spatial crop diversification, three systems were considered: M RB, M S, and M FS. In M RB, maize was cultivated in association with runner bean, *Phaseolus coccineus* L., at 47500 plants ha<sup>-1</sup>, in a row mixed cropping. In M\_S, maize was cultivated in strip mixed cropping with sorghum, Sorghum bicolor (L.) Moench, at 47500 plants ha<sup>-1</sup>. Finally, the flower strip mix cultivated in association with maize in M\_FS consisted of buckwheat, Fagopyrum esculentum Moench (40 kg ha<sup>-1</sup>), phacelia, *Phacelia* sp. (10 kg ha<sup>-1</sup>), and alfalfa (*Medicago* sativa L. (10 kg ha<sup>-1</sup>). In all the mixed cropping systems, a mix of rye and winter pea was cultivated as winter catch crop, presenting therefore both spatial and temporal diversification. Maize was always sown with 95000 plants  $ha^{-1}$  with a row distance of 75 cm, with the exception of the treatment M S with a sowing density of 47500 plants ha<sup>-1</sup> and a row distance between maize and sorghum of 25 cm. An illustration of the spatial arrangement of the crops in the different treatments is given in Figure S2. Different cultivars of maize were cultivated: P8057 in M M, Movanna in M MC and M S, and Ambient in M RB and M FS. This choice was based on current agronomical practices in the area, in order to ensure good combination

and low competitiveness with the companion crop in the mixed cropping systems. Furthermore, treatments differed among each other in terms of field management practices. In the reference system M\_M, the soil was tilled conventionally, only mineral fertilizer was applied, and weeds were managed chemically; on the contrary, in the multiple cropping and mixed cropping systems, soil was tilled with a subsoiling technique, animal organic manure was applied, and weeds were managed mechanically or manually. Having different maize varieties implies also that the timing of many field management practices differed between treatments. Details of field management of each treatment are reported in Table 1.

# **Data collection**

Data were collected in 2019 [(I) 24-26 July and (II) 3-5 September] and 2020 [(III) 15-16 July and (IV) 10-12 August]. The survey periods have been chosen based on the vegetation period of maize and companion crops in each treatment and the period of main activity of the organisms studied. Considering that the aim of the study was to compare different diversification strategies of cropping systems, we conducted the sampling when differences in terms of crop development and soil cover (with particular regards to the companion crops) were noticeable between treatments. The duration of each sampling event was 48h, except for the sampling in July 2020 which was interrupted after 24h due to bad weather conditions. In 2019, the mean temperature measured during the main growing season (April-September) near the experimental site was 14.9 °C, with 304 mm precipitation. In 2020, during the same period the mean temperature was 15.3 °C, with 483 mm precipitation. Monthly temperature and precipitation measured in both years of data collection are reported in Table S1. We measured a set of proxies relevant for the ecosystem function 'biological pest control', following the REFA approach (Meyer et al., 2015). Those proxies included: predation rates on insect baits, attack rates on artificial caterpillars, predation rates on weed seeds, and activity density of the main epigeal predator's taxa. In each plot we placed three sampling points, which consisted of four items to measure the ecosystem functioning proxies investigated (pitfall traps, dummy caterpillars, insect baits, and seed cards). Vertebrates were excluded from seed cards and insect baits using a metal netting with  $1.2 \text{ cm}^2$ mesh, whereas dummy caterpillars were exposed without any exclusion cage.

Predator activity density was measured with pitfall traps buried in the ground. Pitfalls were part-filled with saturated salt solution. We further counted and distinguished the collected individuals among taxa of generalist predators (Carabidae, Araneae, Staphylinidae, Opiliones, Chilopoda). Only Carabidae and Araneae were collected in conspicuous number, therefore, only for these taxa the activity

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Year	Treatment <sup>1</sup>	Sowing and harvest/ mowing date	Crop species and cultivar	Tillage	Fertilization	Weed management	Winter catch crop
2019	M_M	Maize: 7/05–18/09	P8057 (FAO class 210)	Conventional inversion plowing	Mineral fertilization	Chemical: 12/04, 4/06, 9/07	~
	M_MC	Maize: 29/05–18/09	Movanna (FAO class 210)	Subsoiling	Animal organic manure	Mechanical: 4/06, 19/06, 24/06, 2/07	Rye + winter pea
	M_S	Maize: 29/05–18/09 Sorghum: 29/05–19/09	Maize: Movanna Sorghum: DSV16149	Subsoiling	Animal organic manure	Mechanical: 4/06, 19/06, 24/06, 2/07 Manual: 17/07	Rye + winter pea
	M_RB	Maize: 29/05–18/09 Runner bean: 29/05–19/09	Maize: Ambient (FAO class 120) Runner bean: WAV512	Subsoiling	Animal organic manure	Mechanical: 4/06, 19/06, 24/06, 2/07 Manual: 17/07	Rye + winter pea
	M_FS	Maize: 29/05–18/09 Flower strip sowing and mowing date: 29/05–15/07, 1/08	Maize: Ambient Flower strip mix: buckwheat, phacelia, alfalfa	Subsoiling	Animal organic manure	Mechanical: 19/06, 24/06, 2/07 Manual: 17/07	Rye + winter pea
2020	M_M	Maize: 6/05–24/09	P8057	Conventional plowing	Mineral fertilization	Chemical: 7/04	/
	M_MC	Maize: 3/06–24/09	Movanna	Subsoiling	Animal organic manure	Mechanical: 10/06, 16/06, 22/06	Rye + winter pea
	M_S	Maize: 3/06–24/09 Sorghum: 3/06–24/09	Maize: Movanna Sorghum: DSV16149	Subsoiling	Animal organic manure	Manual: 22/06	Rye + winter pea
	M_RB	Maize: 3/06–24/09 Runner bean: 22/06–24/09	Maize: Ambient Runner bean: WAV512	Subsoiling	Animal organic manure	Mechanical: 22/06	Rye + winter pea
	M_FS	Maize: 3/06–24/09 Flower strip sowing and mowing: 3/06–7/07, 22/07	Maize: Ambient Flower strip mix: buckwheat, phacelia, alfalfa	Subsoiling	Animal organic manure	Manual: 22/06	Rye + winter pea
<sup>1</sup> M_M: mai	ize monoculture; M	_MC: maize multiple cropping; M_S: m	aize–sorghum mixed cropping; M_RB: m	aize-runner bean mixed cropping; M	_FS: maize–flower strip mixe	ed cropping.	

**TABLE 1** Cultivated crop species, cultivars, sowing and harvest dates, and management of the investigated treatments during 2019 and 2020.

density was analysed also separately. The duration of 48 h of the sampling allows the assessment of arthropod's activity, whereas it would not be enough to gather specific data at species level.

Predation was assessed using artificial caterpillars made from plasticine. Dummies were 25 mm long and 5 mm wide (Low et al., 2014). After exposure, artificial caterpillars were collected and bite marks were assessed. We identified marks made by arthropods and vertebrates (mammals and birds). Furthermore, we also considered the bite frequency per dummy by arthropods, counting the marks left on each artificial caterpillar.

Insect predation was also assessed measuring the predation rates of insect baits (fly pupae, *Lucilia* sp.) placed on the ground. Predation was calculated for each sampling point as proportion of removed baits. To assess seed predation, the removal rate of the seeds from the plant species *Sinapis arvensis* L., *Capsella bursa-pastoris* (L.) Medik., and *Anthriscus sylvestris* (L.) Hoffm. was calculated.

Furthermore, in 2019 and 2020 weeds were counted twice after emergence, assessing number of weeds, species richness, and weed biomass (biomass data are available only for 2019) (Figure S3, Table S4). Weeds were counted for each plot in one subplot measuring  $0.75 \times 2$  m. The  $1.5 \text{ m}^2$  area covered two rows of maize with the interrow area. Weeds were counted with different timings for the standard and short-season maize varieties (2019: standard maize: 31 May and 2 July; short-season maize: 21 June and 9 July; 2020: standard maize: 9 and 23 June; short-season maize: 23 June and 7 July). In the M\_FS treatment, flower biomass was also measured.

Ten seeds of each species were placed in Petri dishes on the surface of 80 grit sandpaper lightly sprayed with an aerosol glue and exposed on the ground (Westerman et al., 2003). Predation rates were calculated for each seed species separately as proportion of removed seeds. Predation of the smaller seeds *C. bursa-pastoris* could not be considered in September 2019 due to a heavy rain during the sampling which dislocated a lot of those seeds, compromising the reliability of the assessment.

# Statistical analysis

The effects of treatment and sampling date (i.e., explanatory variables) on the ecosystem function proxies were analysed fitting generalized linear mixed models (GLMMs) with the package glmmTMB (Brooks et al., 2017). The models included the fixed effects treatment and sampling date as well as their interaction. Models on weed seed predation did not include this interaction term to ensure a better fit. The factors 'block' and 'plot' were always included as random effects in a nested structure. An observation level random effect was added in the models on insect predation and seed predation to account for overdispersion of the data, whereas the random effect 'sampling point' was added in the models on artificial caterpillar predation, where two dummies were exposed at the same location. For activity density data, negative binomial error distribution (log link function) was used, whereas predation rates (proportional and binary data) were analysed using a binomial error distribution (logit link function). The fit of each model was verified looking at the residuals using the package 'DHARMa' (Hartig & Lohse, 2020). The main effects of our variables and their interaction were tested using the Wald  $\chi^2$  test through the 'anova' function. If significant, a pairwise post-hoc comparison was performed using the 'emmeans' package (Lenth, 2018) and Tukey's test was used to separate the means. All statistical analyses were performed with R v.4.0.5 (R Core Team, 2021).

# RESULTS

In total, >3900 arthropods were collected over both years, 3047 in 2019 and 867 in 2020. The vast majority of arthropods collected were generalist predators (3294). Carabidae were captured in highest number (1959 individuals in 2019, 445 in 2020), followed by Araneae (357 individuals in 2019, 294 in 2020). Staphylinidae, Chilopoda, and Opiliones were also collected among predator taxa, but in lower numbers.

Predation rates on insect baits were generally high (74% overall mean), with the highest predation in July 2019 (78%) and the lowest in July 2020 (67%). Attack rates on dummy caterpillars could be assessed for 98% of the total dummies exposed – 58% of the dummies were attacked by arthropods, 21% by vertebrates, resulting in a total of 65% of dummies showing attack marks. Weed seeds were altogether more predated in 2019 than in 2020. The species *C. bursa-pastoris* had higher predation rates in 2019 (19%) followed by *S. arvensis* (9%) and *A. sylvestris* (6%). In 2020 predation rates were for all three species <4%.

# Activity density of generalist predators

Total predator activity density was significantly lower in M\_M compared to all the mixed cropping systems, but not significantly different to the multiple cropping system (Figure 1A, Table 2). The highest activity density was in M\_S (Figure 1A). Predator activity density differed also between sampling dates, but the interaction treatment\*sampling date was not significant (Table 2). During the first sampling event we found a much higher activity density of predators compared to the other samplings (Figure 1B).

Treatment had significant effects also on the single taxa investigated (Table 3). For Carabidae, significant differences between treatments were observed in three sampling events (II, III, IV), whereas during the first one no differences were found (Table 3). For this taxon, the interaction term treatment\*sampling date was significant (Table 2). For Araneae, on the contrary, this interaction was not significant, but there were significant effects of treatment and sampling date (Table 2). Differences between

FIGURE 1 Activity density (mean no. of individuals/trap) of generalist predators collected with pitfall traps (A) by treatment and (B) by sampling date. M\_M: maize monoculture; M\_MC: maize multiple cropping; M\_RB: maize-runner bean mixed cropping; M\_S: maize-sorghum mixed cropping; M\_FS: maize-flower strip mixed cropping. Different letters capping the treatments in A indicate significant differences between treatments (Tukey's test: P < 0.05). Boxplots show the raw data: they represent the first and third quartile (top and bottom box), the median (line in between), 1.5× the interquartile range (whiskers), and the outliers (dots). The black short horizontal line and vertical bars (within the boxplots) represent the estimated marginal mean ± SE.



treatments were found for all the sampling dates except for sampling II (Table 3).

# Assessment of biocontrol potential

Predation rates on insect prey were affected by treatment and sampling date, whereas the interaction between these two terms was not significant (Table 2). Predation rates averaged over sampling dates were significantly higher in M\_FS compared to the reference system M\_M (Figure 2A). M\_MC, M\_RB, and M\_S had intermediate predation rates, which did not differ, neither among each other nor from the other two treatments. When considering sampling dates separately, in July 2019 we found similar predation rates between systems (Figure 2B), whereas in the sampling events II, III, and IV, predation rates measured in M\_M were lower than in the other treatments, but only in July 2020 (compared to M\_FS) and August 2020 (compared to M\_MC and M\_FS) this difference was statistically significant.

In terms of attack rates on artificial caterpillars by arthropods, we did not find significant effects of treatments. Sampling date and the treatment\*sampling date interaction were both significant (Table 2). In general, attack rates had the highest proportion in M\_FS (73%), and the lowest in M\_M (52%), but this difference was not significant (Figure 3A). When looking at the frequency of attack (i.e., the number of marks left by arthropods on each dummy), all the explanatory variables (treatment, sampling date, and their interaction) had significant effects (Table 2). In particular, the number of attack marks on the dummies was higher on M\_FS, M\_S, and M\_RB than on M\_M and M\_MC (Figure 3B).

Predation on weed seeds was strongly affected by the sampling date (Table S3) – it was much higher in July 2019 than in the other sampling events. This effect was found for all weed species investigated. Differences between treatments were found only for *S. arvensis*. For this species, the mean predation rate averaged over sampling date was higher in M\_FS (5.4%), M\_S (4.7%), and M\_RB (3.3%) than in M\_M (0.5%), whereas in M\_MC (2.3%) it was intermediate (Table S2). Predation rates of *A. sylvestris* and *C. bursa-pastoris* seeds were not significantly affected by treatments (Table S3).

TABLE 2 Effects of treatment, sampling date, and their interaction on the ecosystem service proxies investigated.

Proxies	Explanatory variables	χ <sup>2</sup>	d.f.	Р
Total predator activity density	Treatment (T)	72.1	4	<0.001
	Sampling date (S)	541.6	3	< 0.001
	T*S	16.7	12	0.16
Carabidae activity density	Т	54.2	4	< 0.001
	S	637.5	3	<0.001
	T*S	27.8	12	0.005
Araneae activity density	Т	51.1	4	< 0.001
	S	51.7	3	< 0.001
	T*S	12.6	12	0.39
Predation rates on insect prey	Т	13.7	4	0.008
	S	9.0	3	0.02
	T*S	17.2	12	0.14
Attack rates on dummy caterpillars	Т	4.7	4	0.31
	S	25.9	3	<0.001
	T*S	23.5	12	0.02
Frequency of attack on dummy caterpillars	Т	17.0	4	0.001
	S	134.8	3	< 0.001
	T*S	34.6	12	<0.001

**TABLE 3** Mean (± SE) activity density of all generalist predators and the two main taxa of predators (Carabidae, Araneae) collected with pitfall traps in each treatment (M\_M: maize monoculture; M\_MC: maize multiple cropping; M\_RB: maize-runner bean mixed cropping; M\_S: maize- sorghum mixed cropping; M\_FS: maize-flower strip mixed cropping), during different sampling dates (I: July 2019, II: September 2019, III: July 2020, IV: August 2020).

		Treatment				
Taxon	Sampling date	M_M	M_MC	M_RB	M_S	M_FS
Total predators	I	36.3±4.4a	35.9±4.3a	35.9±4.3a	48.6±5.7a	53.6±6.3a
	II	6.0±1.0a	9.0±1.4ab	11.6±1.7bc	16.3±2.2c	11.0±1.6abc
	Ш	6.9±1.1a	8.2±1.3a	9.9±1.5ab	16.4±2.2b	14.8±2.0b
	IV	5.2±0.9a	5.7±1.0a	9.0±1.4ab	11.9±1.7b	11.6±1.6b
Carabidae	I	30.8±4.1a	31.6±4.2a	30.4±4.1a	41.6±5.4a	41.2±5.4a
	II	2.6±0.6a	5.8±1.0ab	7.4±1.2b	10.6±1.6b	6.4±1.1b
	Ш	$3.4\pm0.7ab$	2.7±0.6a	$3.5\pm0.7ab$	9.7±1.5c	7.0±1.2bc
	IV	$3.6\pm0.7ab$	4.4±0.8ab	6.6±1.1ab	7.9±1.3b	7.3±1.2ab
Araneae	I	3.7±0.8a	2.6±0.6a	$3.5\pm0.7ab$	$5.3 \pm 1.0 ab$	9.8±1.6b
	П	1.2±0.4a	1.3±0.4a	$2.1 \pm 0.5a$	2.1±0.5a	3.5±0.7a
	Ш	2.4±0.6a	4.4±0.9ab	$5.0\pm0.9ab$	$5.5 \pm 1.0$ ab	6.1 ± 1.1b
	IV	1.6±0.4ab	1.2±0.4a	$2.3\pm0.5ab$	3.7±0.8b	4.2±0.8b

Means within a row followed by different letters are significantly different (Tukey's test: P < 0.05).

# DISCUSSION

The present study investigated the effects of different maize cropping systems on the activity and biocontrol potential of generalist predators. Our results showed that management practices and crop diversification at local scale have the potential to affect the activity of natural enemies and the ecosystem service 'biological pest control' they can deliver. Despite the small size of the experimental plots, and considering the high mobility of the investigated organisms, we were able to detect substantial differences between systems investigated. Sampling dates had an effect on all the proxies investigated, whereas an effect of treatment was found for all the proxies except for the attack rates on dummy caterpillars. In general, we found higher activity density of predators in the low-input systems that established a dense and diverse vegetation cover of the ground during the growing season (i.e., maize/ **FIGURE 2** Estimated marginal mean (± SE) predation rates (%) on insect baits (A) by treatment and (B) by sampling date. M\_M: maize monoculture; M\_MC: maize multiple cropping; M\_RB: maize-runner bean mixed cropping; M\_S: maize-sorghum mixed cropping; M\_FS: maize-flower strip mixed cropping. Different letters capping the treatments in A indicate significant differences between treatments (Tukey's test: P < 0.05).



sorghum, maize/flower strips). We also observed that the reference system with maize monoculture and conventional crop management had a lower activity density and biocontrol potential than the mixed cropping systems. However, these effects were not consistent for all the parameters investigated and for every sampling date. For the proxy attack rates on dummy caterpillars, for instance, the difference between treatments was not statistically significant. On the contrary, the number of marks left by predators on each dummy was significantly higher in the three mixed cropping systems than in the other two. Predation rates of insect baits were significantly different only between maize monoculture and maize/flower strips treatments. Differences in activity density of predators were observed between samplings, likely as response to local and climatic conditions. During the sampling in July 2019, many more predators were collected than during the other sampling events. This was mainly due to the extremely high number of carabids, whose activity can be strongly affected by weather variables, in particular temperature. Saska et al. (2013) showed that activity of carabids tends to increase substantially with increasing temperature, in

particular when considering maximum temperature. This seems to be confirmed also in our study, as the daily mean temperature measured around the sampling in July 2019 was higher compared to the other sampling dates. Carabids represent the most important group among the predators collected in this study in terms of abundance. Many species of ground beetles that are omnivorous or herbivorous may also benefit by the presence of enhanced vegetational resources (e.g., flower strips, weeds), which may represent an additional source of food (Lys et al., 1994).

With regards to the spatial diversification practices implemented in the treatments, our results are in line with the hypothesis that enhancing plant diversity, increasing habitat diversity at local scale, can benefit predators (Dassou & Tixier, 2016). In M\_FS, flower strips were tested to enhance biodiversity and offer a better habitat for beneficial arthropods. It has been shown that this measure has the potential to enhance biological control of aphids and provide benefits for natural enemy biodiversity (Albrecht et al., 2021). According to this assumption, we found higher activity of predators and biocontrol potential in M\_FS compared to the reference treatment.



**FIGURE 3** Estimated marginal mean (± SE) (A) arthropod attack rates (%) on dummy caterpillars and (B) number of marks left by arthropods on each dummy, by treatments. M\_M: maize monoculture; M\_MC: maize multiple cropping; M\_RB: maize-runner bean mixed cropping; M\_S: maize-sorghum mixed cropping, M\_FS: maize-flower strip mixed cropping. Different letters within a panel capping the treatments indicate significant differences between treatments (Tukey's test: P < 0.05).

This result can be explained by the flower strips providing not only food resources, but also structural resources to natural enemies. Tschumi et al. (2015, 2016) demonstrated positive effects of tailored flower strips for beneficial arthropods in potato and wheat crops. Although the composition of flower species and flowering times is fundamental for parasitoids (Wäckers & van Rijn, 2012), generalist predators that do not use floral resources as food source may respond to the greater abundance of prey and/or the shelter provided by flower strips (Bianchi et al., 2006). Plant complexity and vegetation structure may also influence predator–predator interactions, diminishing antagonism and thus increasing overall enemy impact on herbivore prey (Finke & Denno, 2002).

Similarly, the mixed cropping system maize/sorghum (M\_S) had positive effects on activity density, being comparable to those obtained in M\_FS and significantly higher than the other treatments, whereas no significant differences were found for predation rates. An explanation for the positive results in M\_S may be found in the weeds biomass present in this treatment, which may have created very similar conditions in terms of soil cover to those in M\_FS. This is also confirmed by the results on

weed biomass. A large body of research shows a positive relationship between high weed cover and densities of predators (Balfour & Rypstra, 1998; Hough-Goldstein et al., 2004; Diehl et al., 2012). This is explained by both structure and source quality effects (Wardle et al., 1999). The presence of arable weeds may foster predator activity density and species richness via resource-mediated effects, such as higher availability of weed-borne resources (e.g., seeds and pollen) and herbivorous prey, and structure-mediated effects altering the microclimate (Diehl et al., 2012). Moreover, another element of habitat complexity in this treatment was given by the reduced row distance between maize and sorghum compared to the maize in the monocultural system, which may have contributed to create more favourable habitat conditions for predators.

On the contrary, the conventional maize cultivation system was characterised by a large area of bare ground, which creates a less favourable habitat for predator arthropods caused by less availability of structural shelter, alternative prey, and food resources. In our experiment, the maize monoculture treatment M\_M was managed under a conventional regime in terms of tillage, fertilization, and weed management compared to the other treatments. It is therefore possible, that the results obtained can be partially explained by the different field management practices. Management practices have the potential to influence directly and indirectly the presence and activity of natural enemies in the crop field. In conventional maize cultivation, inversion tillage and chemical weed control are often used to minimize the competition between crop and weeds at the beginning of the growing season (Hall et al., 1992). Both chemical and mechanical methods to control weed flora have the potential to influence weed communities both in abundance and composition, thereby affecting above-ground arthropods. For example, negative effects are mainly related to the destruction of the microhabitat and the consequent loss of shelter and food resources, causing natural enemies to emigrate from the field (Ekschmitt et al., 1997; Thorbek & Bilde, 2004). The difference in soil cover between M\_M and both M\_S and M\_FS becomes more evident looking at the data on weed count and biomass. Lower activity density of predators compared to the mixed cropping treatments M S and M FS was found also for the multiple cropping system (M\_MC), which was managed with the same low-input regime as the mixed cropping systems. Here, it is important to mention that the mechanical weeding in M MC was effective in keeping a low weed pressure similar to that in M\_M). In the mixed cropping systems, on the contrary, the spatial arrangements of the crops made the mechanical weeding more difficult and less effective (even in combination with manual weeding), resulting in much higher weed pressure in particular in M S. Additionally, in 2020, mechanical weeding in M\_MC was carried out 3×, compared to once in the other low-input treatments. Therefore, it seems that the microhabitat conditions created by the vegetation cover within the field may be the driving factor in affecting generalist predator's activity. However, further studies on specific aspects of crop management are necessary to disentangle the effects of different practices implemented, as the mechanisms involved are extremely complex.

Natural enemies may be influenced also by the fertility management. In particular, using animal and green manures instead of mineral fertilizer may increase the availability of soil organic matter, subsequently influencing the soil-based food web and the predator activity (Eyre et al., 2009; Rowen et al., 2019). The presence of Collembola, for instance, which represent an important component of generalist predators' diet, may be fostered by organic fertilization and crop residues left after cover crop cultivation, leading to an increase in epigeal predators if present in high densities (Bilde et al., 2000; Birkhofer et al., 2008). In this experiment, the subsoiling technique applied in the low-input treatments may have helped to preserve the structure of the top soil, in contrast to the inversion tillage in M\_M, favouring a more diverse and abundant detritivore community or an enhanced

microclimate. Ploughing, in fact, represents one of the major causes of disturbance affecting the activity of beneficial arthropods on the ground (Thorbek & Bilde, 2004).

We also found that mowing of the flower strip affected the activity of predators. Horton et al. (2003), in a study investigating the effects of mowing in an orchard, showed that the frequency of mowing affects the density of beneficial and phytophagous arthropods and predators increased in number in association with decreased mowing events. In our study, we found a significant shift in the pattern of activity density of generalist predators in M FS between July and September 2019, which could be related to the mowing of flower strips really close down to the soil in August 2019. However, this difference was statistically significant only for spiders, which have been shown to be more sensitive to grass cutting than other predators (Thorbek & Bilde, 2004). A possible explanation for this effect is that web spiders take advantage from the structural support offered by a dense vegetation cover to anchor their webs (Uetz, 1991; Birkhofer et al., 2007). Removing this structural support from the field likely made habitat conditions for these predators less suitable.

In conclusion, our study shows that local crop habitat heterogeneity and management practices within field affect generalist predators, thus their biological control potential of pests. In maize cropping systems, habitat heterogeneity may be achieved not only by implementing strategies of crop diversification at spatial and temporal scale, but also by applying conservative and low-input crop management practices. Reduced management of weeds with less dependence on chemical herbicides, reduced tillage, and cultivation of cover crops are all practices that, in combination with a more diversified cropping system, have the potential to influence the habitat within field for natural enemies. In order to disentangle the effects of the single management practice, specific investigations may be carried out decoupled from other factors. In this study, the experimental design does not allow such separation, as the cropping systems are observed as a whole. Nevertheless, it seems that low-input mixed intercropping maize systems have the potential to support the delivery of ecosystem services such as biological control compared to a conventional monocultural maize cropping system. Future research should aim at investigating more sustainable maize systems that support biodiversity and delivery of ecosystem services, while maintaining high production levels.

# **AUTHOR CONTRIBUTIONS**

Giovanni Antonio Puliga: Conceptualization (equal); data curation (lead); formal analysis (lead); writing - original draft (lead); writing – review and editing (lead). Timo Sprangers: Data curation (supporting); resources (equal); writing review and editing (supporting). Hilfred Huiting: Data curation (supporting); resources (equal); writing - review and editing (supporting). Jens Dauber: Conceptualization (equal); project administration (lead); supervision (lead); writing - review and editing (supporting).

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# **CONFLICT OF INTEREST**

The authors have no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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#### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Figure S1.** (A) Maize monoculture, (B) maize multiple cropping, (C) maize-runner bean mixed cropping, (D) maize-sorghum mixed cropping, and (E) maize-flower strip mixed cropping plots at the field experiment during the sampling in July 2020.

**Figure S2.** Spatial arrangement of the crops in the various treatments. Maize monoculture and maize multiple cropping (above left), maize–runner bean mixed cropping (above right), maize–sorghum mixed cropping (below left), and maize–flower strip mixed cropping (below right).

**Figure S3.** Weed biomass (kg ha<sup>-1</sup>) measured in 2019. M\_M: maize monoculture; M\_MC: maize multiple cropping; M\_RB:

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maize-runner bean mixed cropping; M\_S: maize-sorghum mixed cropping; M\_FS: maize-flower strip mixed cropping. In M\_FS weed and flower biomass were calculated separately. **Table S1.** Monthly precipitation (mm) and temperature (°C) in 2019 and 2020 measured at the local weather station near the field site.

**Table S2.** Mean ( $\pm$  SE) predation rates (%) of the three weed species averaged over the sampling dates. M\_M: maize monoculture; M\_MC: maize multiple cropping; M\_RB: maize-runner bean mixed cropping; M\_S: maize-sorghum mixed cropping; M\_FS: maize-flower strip mixed cropping. Means within a row followed by a different letter are significantly different (Tukey's test: P < 0.05).

**Table S3.** Effects of treatment and sampling date on the predation rates of the three weed species investigated. P-values are based on  $\chi^2$  tests.

**Table S4.** Mean ( $\pm$ SE) weed count (no. m<sup>-2</sup>), species richness (no. m<sup>-2</sup>), and biomass (kgha<sup>-1</sup>) measured in

2019 and 2020. Weeds were counted twice each year after emergence. Weed biomass data are available only for 2019. M\_M: maize monoculture; M\_MC: maize multiple cropping; M\_RB: maize-runner bean mixed cropping; M\_S: maize-sorghum mixed cropping; M\_FS: maize-flower strip mixed cropping. In M\_FS also the flower biomass was measured. In 2020 weed count and species richness data are not available for treatments M\_RB and M\_S.

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