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Structure, relationships and diversity in the community of aphids and aphidophagous species in alfalfa

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Abstract

There is a need for comprehensive research on the species structure and the population dynamics of the most common aphidophagous species. A critical factor of the effectiveness of aphid bio-control is the ratio of beneficial polyphagous (generalist) to oligo- or monophagous (specialist) species within the various trophic groups. Aphids' population density and environmental conditions influence the development and potential feeding of useful insects. The present study aimed to determine the community structure, relationships and diversity between aphids and their aphidophagous species in alfalfa fields using the following methods: sweeping with an entomological net, the quadratic method, coloured sticky board method, route survey method and visual observations. Research on the structure of the aphid–aphidophagous community revealed that aphidophagous species belong to three groups: (1) polyphagous predatory bugs from the families Anthocoridae and Nabidae, (2) oligophagous and polyphagous predators from the families Coccinellidae, Syrphidae and Chrysopidae; and (3) monophagous and oligophagous parasitoids, primarily from the families Aphidiidae and Ichneumonidae. From mid-May to June, there was a sufficiently large potential for aphidophagous species (Coccinellidae, Syrphidae, Chrysopidae, Anthocoridae and Nabidae) to control aphids, while in September, predatory ladybirds from the Coccinellidae family were the main biological control agents. Coccinellidae (Coleoptera) exhibited the highest values of diversity, dominance and richness indices among insect groups in the aphid–aphidophagous community. The existence of diverse aphidophagous species in alfalfa fields suggests that these predators can complement each other, leading to effective biological control of aphids. The synergy among different predator species holds promise for enhancing the overall efficacy of integrated pest management strategies.

Introduction

Food webs focused on aphids (Hemiptera, Sternorrhyncha) consist of intricate networks of species. Some relationships are directly related to prey traits such as the species and density (Allahyari *et al.*, 2004), the prey's ability to defend themselves and toxicity (Rotheray, 1989). Other relationships are mediated through the host plant characteristics, such as the presence of secondary metabolites (Amiri-Jami *et al.*, 2016). These trophic interactions can become even more complex with the inclusion of predators and parasitoids within aphid colonies (Amiri-Jami *et al.*, 2017). Diverse and intricate relationships have been pivotal in the development and maintenance of species diversity, not only in alfalfa but also within the broader biodiversity of agricultural systems (Putnam *et al.*, 2015). Studying these interactions led to considerable theoretical progress in ecology and evolutionary biology (Dyer *et al.*, 2010). Most biodiversity studies have focused on individual species lists and known diversity theories yet often overlook trophic interactions and species richness (Dyer *et al.*, 2010). It is challenging for individual studies to establish these complex relationships in alfalfa, but aggregating such studies could contribute to a more comprehensive understanding. For this purpose, it is necessary to have an in-depth knowledge of insect pests and their parasitoids and predators.

The most significant and widespread interaction among aphidophagous species is with their aphid prey. The occurrence and abundance of aphids have a considerable impact on the populations of ladybirds, with predatory ladybird populations generally considered 'bottom-up' regulated rather than 'top-down' (Dixon, 2000; Hodek *et al.*, 2012).

The relationship between predator diversity and biological control efficacy can vary depending on the system and species composition. Such variation can be influenced by specific traits of predators and prey (Tylianakis and Romo, 2010) or by interactions within the predator community (Straub and Snyder, 2006). Furthermore, predator diversity may positively affect aphid biological control due to niche interdependence (Straub *et al.*, 2008). Therefore, examining the relationships within the aphid–aphidophagous agro-ecosystem and assessing the combined or individual predatory effects on the aphid population is crucial.

Aphids are major pests that damage on crops by feeding on phloem, producing honeydew, and transmitting viruses, causing serious economic losses (Kumar, 2019). In the early stages of infestation, aphid adults are wingless. However, as the population increases, winged forms appear in subsequent generations to facilitate dispersal from one plant to another (Braendle *et al.*, 2005). Aphids are typically found on the growing points of host plants, including tips, flowers and developing pods, and can cover the entire plant at high densities (Blackman and Eastop, 2000). Although many insecticides are recommended for pest control, they can be detrimental to beneficial organisms, leave residues and pose risks to humans, animals and the environment (Lorenz, 2009). Employing aphidophagous species in biological control is an effective strategy for managing invasive aphid populations. Ladybirds are predatory insects that feed on a wide food variety, with aphids constituting their primary prey. The seven-spotted ladybird (*Coccinella septempunctata*) (Coleoptera: Coccinellidae) is a well-known predator of aphids within the ladybird group. Both the larvae and adults of *C. septempunctata* prey on aphids, with consumption rates being higher in females than in males (Khan and Yoldaş, 2018). Predation rates of the seven-spotted ladybird increase with developmental stage (Ali and Rizvi, 2007). However, coccinellids' development and feeding habits are influenced by changes in environmental conditions and aphid population dynamics (Sloggett, 2021). According to the author, the diversity of Coccinellidae and their adaptation to various habitats make them significant biological control agents with economic implications.

The Syrphidae family (Diptera) is highly beneficial as a predator of economically important aphids, contributing to population reduction (Wojciechowicz-Żytko and Wilk, 2023). Syrphids are natural enemies of insects, with substantial biocontrol potential and field applications (Jiang *et al.*, 2024). Selecting third-instar larvae for the aphid control in field applications was recommended (Jiang *et al.*, 2023). Unlike ladybirds, such as *Harmonia axyridis* (Coleoptera: Coccinellidae) and *C. septempunctata*, predatory flies of the Syrphidae family do not undergo summer diapause, making them more suitable as predators during the summer (Ohashi *et al.*, 2003), especially when ladybirds are less active.

Syrphid flies provide two ecosystem services: biological pest control and pollination (Ssymank *et al.*, 2008; Omkar, 2016; Dunn *et al.*, 2020). Adult hoverflies, which are the second most important pollinators in the world after bees (Rader *et al.*, 2016, 2020; Wotton *et al.*, 2019), feed on pollen and nectar. In Bulgaria, Harizanova (1989), Harizanov and Babrikova (1990) and Harizanov *et al.* (1996) conducted extensive studies on the species composition, biology and regulatory capabilities of the most common predator species in the aphid population in alfalfa. Some recommend the cultivation of flowering nectarous plants and annual and perennial grasses in a neighbouring area to increase the impact of ovum-eating parasitoids and other oligophagous and polyphagous predators and parasitoids (Harizanov *et al.*, 1996). Authors in Bulgaria have identified 40 species of ladybirds, but not all of them have been studied (Harizanov and Babrikova, 1990).

Previous studies found *Nabis pseudoferus* (Hemiptera: Heteroptera) to be the dominant species among predatory plant bugs (29.6%) (Popova, 1966). Similar results were reported for predatory Heteroptera on aphids in alfalfa by Ivanova (2004) and Atanasova (2006). Given that studies on aphid predators and parasitoids in Bulgaria were conducted many years ago, there is a pressing need to update and supplement this information. In-depth research on the species structure and population dynamics of the most common aphidophagous species is essential. An important determinant of the effectiveness of aphid biocontrol is the ratio of beneficial polyphagous (generalist) to oligo- or monophagous (specialist) species within the different trophic groups (Hawro *et al.*, 2015). The population density of aphids and environmental conditions influence the development and potential feeding of beneficial insects.

The present study aimed to elucidate the structure, relationships and diversity within the community of aphids and their aphidophagous species in alfalfa.

Materials and methods

In alfalfa, sown spring in 2015, I aim to establish the structure and dynamics of aphids and aphidophagous community. The crop was cultivated under non-irrigated conditions following a preceding oat crop for 4 years (2015–2018). Following alfalfa forage technology (Radeva *et al.*, 2006) forage was harvested at the onset of the flowering stage across four regrowth periods during the growing season (first cut in the second half of June, second cut in the second half of July, third cut at the end of July and fourth cut in mid-September).

The community structure of aphids and their aphidophagous species was determined using the following methods:

1. Sweeping with an entomological net, samples were taken once a week throughout the growing season. The entomological bag consisted of a nickel-plated hoop of 30 cm in diameter, with a reinforced heavy-duty sailcloth band, a depth of 80 cm and a handle length of 120 cm. Each sample for a given week included eight replicates, taken diagonally, and covered 20 sweeps (Mihailova *et al.*, 1982). This method was executed during warm and sunny weather, typically between 9 and 11 am, and is considered the most suitable for studying insects found in the vegetative parts of plants (Popova, 1966; Bournoville, 1978). Each sample comprised the collected individuals by 20 sweeps with an entomological net. Collected individuals were transferred and put away in dark-coloured glass vials pre-filled with 70% ethyl alcohol. They were named with the date, month and year and replicated for the respective sample. The collected material was processed in the entomology laboratory at the Institute of Forage Crops, Pleven, Bulgaria.

   Species classification into orders, families and genera is based on various morphological characteristics of adults. Taxonomic keys for identification included distinctive traits such as wing pairs, insect mouthparts, and antenna types. Male genitalia structure and female ovipositor features were the most reliable traits for species identification, especially within the Hymenoptera order (Huber, 2017);
2. The coloured sticky board method involved placing yellow sticky boards, measuring 13.0×25 cm, within the study area, which were replaced weekly. This method helped identify the species composition of aphids and some yellow-attracted aphidophagous insects (Hymenoptera, Heteroptera).
3. The route survey method and visual observations were used to identify parasitised aphids, which were distinguishable from non-parasitised ones by their oval shape and lighter, straw-yellow colour. Once a week, larval and adult forms were collected, along with the leaves. The material was then

placed in cloth bags. The samples were then transferred to the laboratory and grown in glass Petri dishes covered with cheesecloth until adults of the parasitoid emerged from the host's body. Determinations at the family level were made using relevant keys (Harizanov and Babrikova, 1990; Harizanov *et al.*, 1996). In addition to the described method, larvae and pupae of flies from the Syrphidae family were collected and reared in a laboratory environment to identify the parasitoids within them. Syrphidae larvae and pupae were collected, along with the leaves from the field, transferred to the laboratory, and grown in glass Petri dishes covered with cheesecloth until the parasitoid imago emerged from the host's body. Individuals of the family Ichneumonidae and Pteromalidae were found to parasitise flies of the family Syrphidae.

Meteorological factors, such as temperature, relative humidity and precipitation affecting aphid and aphidophagous abundance, were obtained from a Pleven meteorological station. The different weather conditions in individual years determined aphid population densities. The high average day-night air temperature during 2016 (on average exceeding that of the last 20 years by 1.4°C) and the moderately high relative humidity (fig. 1) contributed to the development of aphids, which also determined their high numbers. The higher temperature in 2015 also favoured faster reproduction and development of the aphid colonies.

The preprocessing of the data (analysis of variance (ANOVA)) was carried out before performing the one-way ANOVA. The difference in the number of various insect species was compared by one-way examination of change (ANOVA). Means were compared using the Tukey test at a significance level of 5% ($P \leq 0.05$). Insect group differences across the 4-year study were compared using a *t*-test with the R 'dplyr' package (Wickham *et al.*, 2023). A regression model was used to establish a relationship between an independent variable and a dependent variable by defining a function. Performing a regression analysis helps predict the impact of the aphid density on their aphidophagous species. The statistical analysis of the data was performed through the Statgraphics Plus software (Ziegel and Polhemus, 2000).

The analysis focused on the climatic parameters between March and September of each year to study the occurrence of aphids and their predators in the field. Canonical correspondence analysis (CCA) was conducted to determine the impact of selected climatic variables on the population density of the species. The calculation was performed using the Paleontological Statistics Software Package (PAST) (Hammer *et al.*, 2001).

The study's diversity aspect was α-diversity, representing the species diversity within the community of aphids and their aphidophagous predators over the 4 years. The Shannon–Wiener diversity index (1949) was employed for diversity calculations:

$$H = -\sum [(\mathrm{Pi}) \times \log\,(\mathrm{Pi})] \tag{1}$$

where H = Shannon diversity index; Pi = S/N, where S = number of species, N = total number of individuals; $\sum$ = sum symbol; log = usually the natural logarithm. The natural logarithm has the number e ≈ 2.718 as its base.

Pielou's Evenness Index (E) was used to calculate the evenness of species (Pielou, 1966).

$$E = H/\mathrm{In}\ S \tag{2}$$

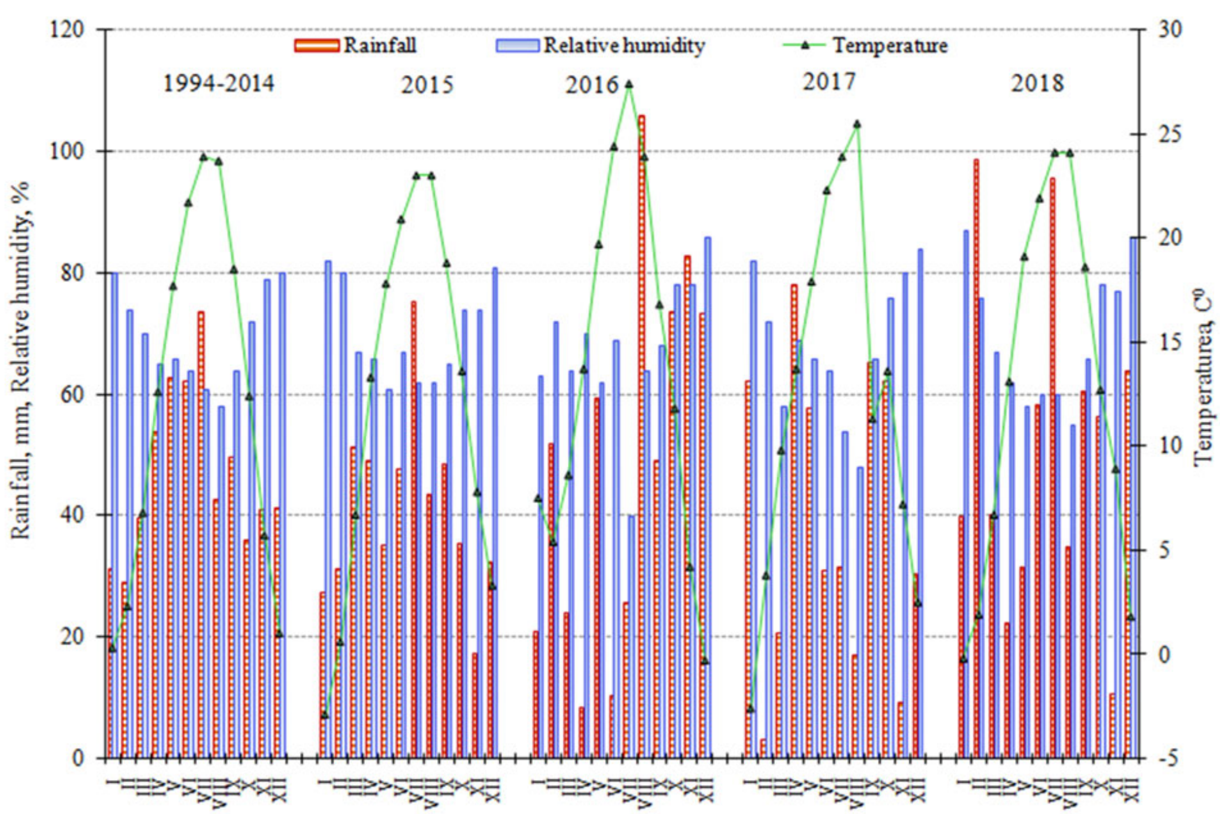

Figure 1. Meteorological characteristics for the region of Pleven, 1994–2018.

where H = Shannon–Wiener diversity index; S = total number of species in the sample; ln = the natural log.

Simpson Index of Dominance ($1/D$) (Simpson, 1949):

$$1/D = 1/[S \sum i(ni/N)^2] \quad (3)$$

where n_i = the number of individuals in species i, N = total number of individuals of all species, $n_i/N = p_i$ (proportion of species individuals i) and S = species richness.

The Margalef's Species Richness Index (d) (Margalef, 1958) is calculated using this formula:

$$d = (S - 1)/\ln N \quad (4)$$

where S = the number of species, and N = the total number of individuals in the sample.

Effective Number of Species (True Diversity): This involves converting diversity indices into true values to provide an accurate conception of diversity at specific sites. The Shannon diversity index, also known as the Effective Number of Species or the Shannon Effective Number of Species, is calculated using the formula developed by Jost *et al.* (2006):

$$\mathrm{ENS} = \exp H \quad (5)$$

where H = Shannon–Wiener diversity index; Exp = exponential function calculation.

Results

Over the 4 years from 2015 to 2018, a comprehensive collection of 25,902 individuals (encompassing both larval and adult stages), was amassed to analyse the community structure of aphids and their aphidophagous species (table 2). The collection included 23,729 aphid individuals (Hemiptera: Aphididae), 265 predatory bugs (Hemiptera: Heteroptera, Anthocoridae and Nabidae), 148 individuals from the Chrysopidae family (Neuroptera), 1076 individuals from the Coccinellidae family (Coleoptera), 345 individuals from the Syrphidae family (Diptera) and 339 parasitoids from the Hymenoptera order. ANOVA results (table 1) indicated that differences were significant at $F = 0.003$.

During alfalfa vegetation, the activity of the individual components in the aphid–aphidophagous association and the population dynamics of the species were monitored by constant weekly sampling.

Two primary factors, namely food availability and climatic conditions, influence the life cycle of aphids. Significantly the highest abundance of aphids occurred in 2016 ($F_{3,7} = 23.673$; $P = 0.038$) (table 2). The conditions in 2015 were also favourable for the development of aphids. During the study period, aphids in alfalfa plants were represented by one family (Aphididae) and five species, with the spotted alfalfa aphid, *Therioaphis trifolii*, being the most abundant species (63.3%) (15,021 individuals). The proportion of the pea aphid (*Acyrthosiphon pisum*, 28.8%) (6834 individuals) was also considerable. Less commonly observed was *Aphis fabae* Scopoli, accounting for 6.2% and ranging between 5 and 15% of all aphids. *Aphis craccivora* Koch and C.L. comprised 1.6%, while *Macrosiphon avenae* Fabricius was scarcely present at 0.1%. Instances of *M. avenae* were occasionally detected, potentially due to its proximity to neighbouring oat crops.

The predatory insect activity was also affected by weather conditions and aphid population dynamics. The densities of various aphidophagous groups were reciprocally related to aphid density. Due to the aphid outbreak in 2016, the participation of aphid predators (namely Coccinellidae, Syrphidae, Chrysopidae, Anthocoridae and Nabidae) was the highest in 2016, followed by 2015 ($F_{3,7} = 32.901$; $P = 0.03417$, table 2). There were predators and parasitoids among the natural aphid pests. Predatory insects, including Coccinellidae, Syrphidae, Cecidomyiidae and Chrysopidae were dominant within the ecosystems, while aphid parasitoids from Aphidiidae (aphidophagous) were encountered only sporadically. A complete analysis of the material, categorised by year, is presented in table 2.

CCA was employed to elucidate the intricate relationships between aphid and predator population densities and climatic factors (fig. 2). Aphid populations exhibited positive correlations with temperature and humidity, whereas rainfall contrarily affected their abundance.

In the biplot, the direction and length of the arrow for each environmental variable indicated its correlation with the axes and its relative importance, respectively. Short arrows, such as that for rainfall, signified a minimal impact on aphid density, around −0.2. Long arrows indicated that the temperature and relative humidity had a strong and positive influence on the aphid population.

Predator density was negatively correlated with aphids, supporting the hypothesis that as their numbers increased, the participation of their prey decreased. Predators can be very effective bioregulators in biological control.

On the other hand, the length of an environmental arrow in a biplot indicated the importance of the variable and how well its values were displayed. It was equal to the maximum rate of change of the variable. Short arrows represented variables with little effect on aphid density, such as rainfall. The factors, having the greatest impact on the population were temperature and relative humidity.

The community structure of aphids and their associated predators and parasitoids was found to be diverse and complex, comprising three main insect groups:

1. Polyphagous predatory plant bugs from the families Anthocoridae (*Orius* spp.) and Nabidae (*Nabis* spp.) (Hemiptera: Heteroptera);
2. Oligophagous and polyphagous predators from the Coccinellidae (Coleoptera), Chrysopidae (Neuroptera) and Syrphidae (Diptera) families;
3. Parasitoids of the order Hymenoptera forming a complex of families (Aphelinidae, Aphidiidae, Pteromalidae and Ichneumonidae).

Table 1. Analysis of variance

Source	The degrees of freedom	Sum of squares	Mean square	*F*-ratio	Significance *F*
Regression	10	38.062	6.169	38.670	0.0030
Residual	1	6.938	2.634		
Total	11	45.000	2.967		

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Table 2. Taxonomic groups of the collected entomological material

Taxonomic groups	2015	2016	2017	2018	Total	%
Aphididae (Hemiptera, Sternorrhyncha)	8315 c	8530 d	2734 a	4150 b	23,729	91.6
Aphidiidae*	19 b	24 c	12 a	11 a	66	0.3
Anthocoridae (Hemyptera, Heteroptera)	19 b	34 c	3 a	3 a	59	0.2
Nabidae (Hemyptera, Heteroptera)	55 b	62 c	54 b	35 a	206	0.8
Chrysopidae (Neuroptera)	61 d	50 c	13 a	24 b	148	0.6
Coccinellidae (Coleoptera)	105 a	609 d	166 b	196 c	1076	4.2
Syrphidae (Diptera)	25 a	156 c	88 b	76 b	345	1.3
Aphelinidae*	10 c	24 d	7 b	4 a	45	0.2
Pteromalidae*	5 a	28 c	17 b	6 a	56	0.2
Ichneumonidae*	20 a	49 b	55 c	48 b	172	0.7
Total	8 634 c	9566 d	3149 a	4553 b	25 902	100.0

*Parasites from families of the Hymenoptera order.

Predatory plant bugs belong to the polyphagous predator group. The most abundant predatory species were *Nabis ferus*, *N. pseudoferus*, *Orius horvathi* and *O. niger*. *Nabis* and *Orius* predators were significantly dominant in 2016 when their prey was at its highest density, followed by 2015 during the 2015–2018 period ($F_{3,7} = 4.515$; $P = 0.039$; $F_{3,7} = 2.174$; $P = 0.043$, respectively, table 2).

The oligophagous and polyphagous predator category, including species from the Coccinellidae, Chrysopidae and Syrphidae families, played a decisive role in regulating aphid populations. The family Coccinellidae, in particular, dominated this trophic level, with its species accounting for over two-thirds of the total predatory count. Notable synchrony was found between the numbers of predators and their prey across the 4 years, with coccinellids having the highest significant participation in 2016 as aphids ($F_{3,7} = 7.373$; $P = 0.040$, table 2).

Coccinellidae was represented by eight species, with *C. septempunctata* and *Hippodamia variegata* (Goeze, 1777) being the abundant and dominant species, accounting for up to 79.5% of the total number of predators at this trophic level (table 3). The ladybird species *C. septempunctata* and *H. variegata* are polyphagous. Therefore, many aphidophagous ladybird species are the most important predators in biological pest control. However, numerous ladybird and variegated ladybird pupae in the aphid colonies of *T. trifolii* and *A. pisum* strongly suggested that these species were acceptable prey for ladybirds in the alfalfa fields under study.

The family Syrphidae was composed of three syrphid species, less numerous than the Coccinellidae. Within the dipteran aphidophagous, two species were particularly dominant (table 4). *Syrphus ribesii* had the highest proportion with more than 50% and was alfalfa's most important syrphid species. *Eupeodes corollae* was almost twice as abundant, whereas *S. vitripennis* was infrequent, often migrating from neighbouring crops or nearby trees. Syrphidae species had the highest significant number in 2016 ($F_{3,7} = 24.548$; $P = 0.018$, table 2).

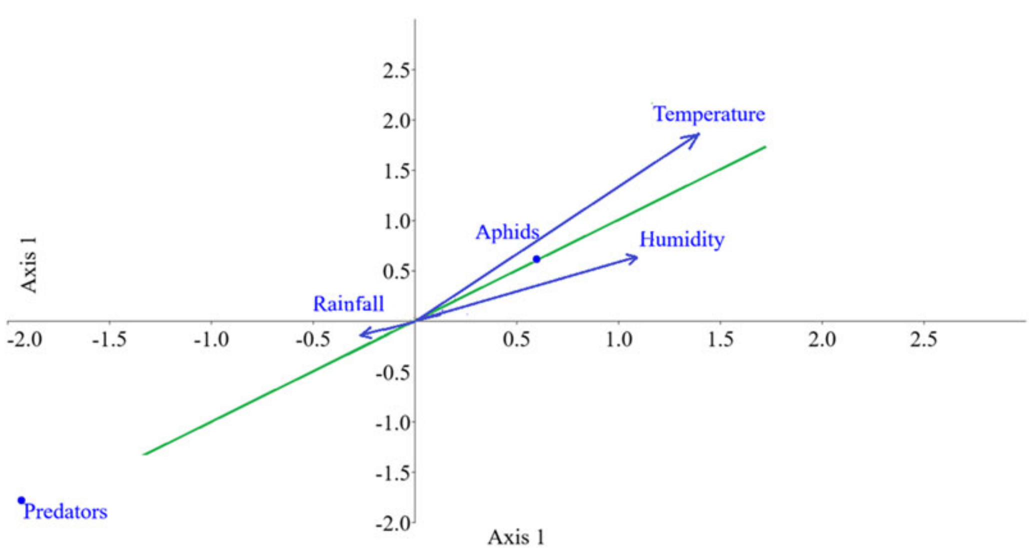

Figure 2. CCA graph based on the correlation of population density of aphids and predators for *Medicago sativa* L. according to several climatic parameters. The period analysed was from March to September.

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Table 3. Percentage ratio between representatives of the family Coccinellidae (Coleoptera) (average for 2015–2018)

Coccinellidae	% Participation
Hippodamia (Hippodamia) variegata Goeze, 1777	32.3
Coccinella (*Coccinella*) *septempunctata* Linnaeus, 1758	47.2
Coccinella (Coccinella) quinquepunctata Linnaeus, 1758	1.9
Propylaea quatuordecimpunctata Linnaeus, 1758	7.3
Thea vigintiduopunctata Linnaeus, 1758[a]	0.2
Scymnus frontalis quadrimaculatus Hrbst, 1783	8.4
Scymnus (*Scymnus*) *frontalis* Fabricius, 1787	1.3
Scymnus (*Pullus*) *subvillosus* Goeze, 1777	1.4

[a]*Thea vigintiduopunctata* Linnaeus, 1758, synonym *Psyllobora vigintiduopunctata* (Linnaeus, 1758).

Three species represented the family Chrysopidae, and the conditions probably determined the species composition of the lacewing during the year and the composition of the neighbouring cultures. Only the predominant species *C. carnea* (88.5%) was consistently present in the alfalfa crop over the years, achieving the highest number in 2016 ($F_{3,7} = 2.977$; $P = 0.007$). The other two species were less frequent within the agroecosystem (table 5).

The development of the Syrphidae aphidophagous community followed a specific pattern: in the first and second years, two dominant species, *E. corollae*, and *Syrphus vitripennis*, comprised up to 44% of the total community. In the third year, *S. ribesii* became the dominant species, with its proportion remaining relatively stable. The alfalfa habitat demonstrated increased diversity, resulting in a more varied species composition and balanced relationships in 2018.

Table 6 presented the findings on the dominance, diversity and evenness of aphids and their aphidophagous predators over the 4 years. The Coccinellidae (Coleoptera) exhibited the highest Shannon diversity index, Simpson's dominance index and effective number of species among the insect groups. The diversity index, commonly used to characterise species diversity in a community, respectively aphid community, ranged from 1.31 to 1.77, with the highest value observed in 2018, followed by 2017. Coccinellidae had the highest diversity and included the most types, reaching up to eight species among the various family levels. Similarly, the dominance index, quantifying the dominance of one or few species in a community, was the highest in 2018, followed by 2017, and fluctuated between 2.75 and 4.98. Higher values indicate higher dominance, with *C. septempunctata* being the most dominant coccinellid species, followed by *H. variegata*. Despite their higher diversity index, the Coccinellidae showed less evenness compared to predatory bugs (Anthocoridae and Nabidae) in 2015 and 2016. Predatory bugs also displayed high dominance, Shannon diversity index and an effective number of species, peaking in 2016 (2.51, 1.12 and 3.07, respectively), followed by 2015. The highest dominance had *N. ferus* exceeded three and a half times the following dominant species *O. niger*. In contrast, they exhibited lower species richness (four species) compared to ladybirds.

Table 4. Percentage ratio between species of the family Syrpliidae (Diptera) (average for 2015–2018)

Syrphiidae	% Participation
Syrphus ribesii Linnaeus, 1758	56.1
Eupeodes corollae Fabricius, 1794	31.2
Syrphus vitripennis Meigen, 1822	12.7

Table 5. Percentage ratio between representatives of the family Chrysopidae (Neuroptera) (average for 2015–2018)

Chrysopidae	% Participation
Chrysoperla carnea Stephens, 1836	88.5
Chrysopa phyllochroma Wesmael, 1841	7.3
Chrysopa commata Kis and Újhelyi, 1965	4.2

The Margalef richness index (d) was lowest for the Chrysopidae and Syrphidae families (0.22) over the years, due to the lowest number of species within each family (three species each). Nonetheless, the Chrysopidae family had a higher diversity and dominance index than the Syrphidae.

Aphids held an intermediate position concerning species richness (five species), effective number of species and diversity index, but displayed the least evenness (except in 2017). Evenness is the homogeneous characteristic and measure of the relative abundance of the different species in the same area. Aphid species were unevenly distributed in the alfalfa as *T. trifolii* quantity within a community was disproportional to other aphids and had the highest abundance.

The analysis of trophic structure was based on species dominance from five aphidophagous families: Chrysopidae, Coccinellidae, Syrphidae, Nabidae and Anthocoridae. The trophic interactions between the dominant aphid and its predators and parasitoids are illustrated in Picture 1.

The occurrence and population dynamics of *T. trifolii* and *A. pisum* varied annually, influenced by climatic conditions. The timing of the mass emergence of aphid species was repeated over the years, with peak densities remaining relatively stable. *Therioaphis trifolii* peaked during the second regrowth, while *A. pisum* peaked during the fourth regrowth. Both species increased their population densities during the fourth and first regrowth, respectively. Typically, the first generation of aphids emerged in late April or early May, followed by a rapid increase in numbers. The summer peak in aphid abundance generally occurred from the beginning of June until the second regrowth, usually by the second 10 days of the month (fig. 3). During the summer period (July and mostly August), there was a sharp decrease in the aphid abundance, which had a different duration depending on the specific weather conditions of the year. After mid-August, a second increase in aphid density occurred, culminating in peak values from the end of the month to the second 10 days of September. Two phenotypes of pea aphids, green and red, appeared concurrently, with the green form being the more prevalent.

The appearance of adult predators and oligophagous parasitoids overlapped or coincided with the aphid appearance, covering the entire study period from May to September. Secondary parasitoids exhibited varied reactions, maintaining more or less similar density throughout the period (May to September), with a slight peak in August. Chrysopidae predominated in August and covered the aphid abundance period at the end of the month. Anthocoridae and Nabidae predatory bugs were present throughout the growing season, and their mass appearance in June coincided with aphid reproduction during that period.

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Table 6. Diversity indices of insect species between the four years

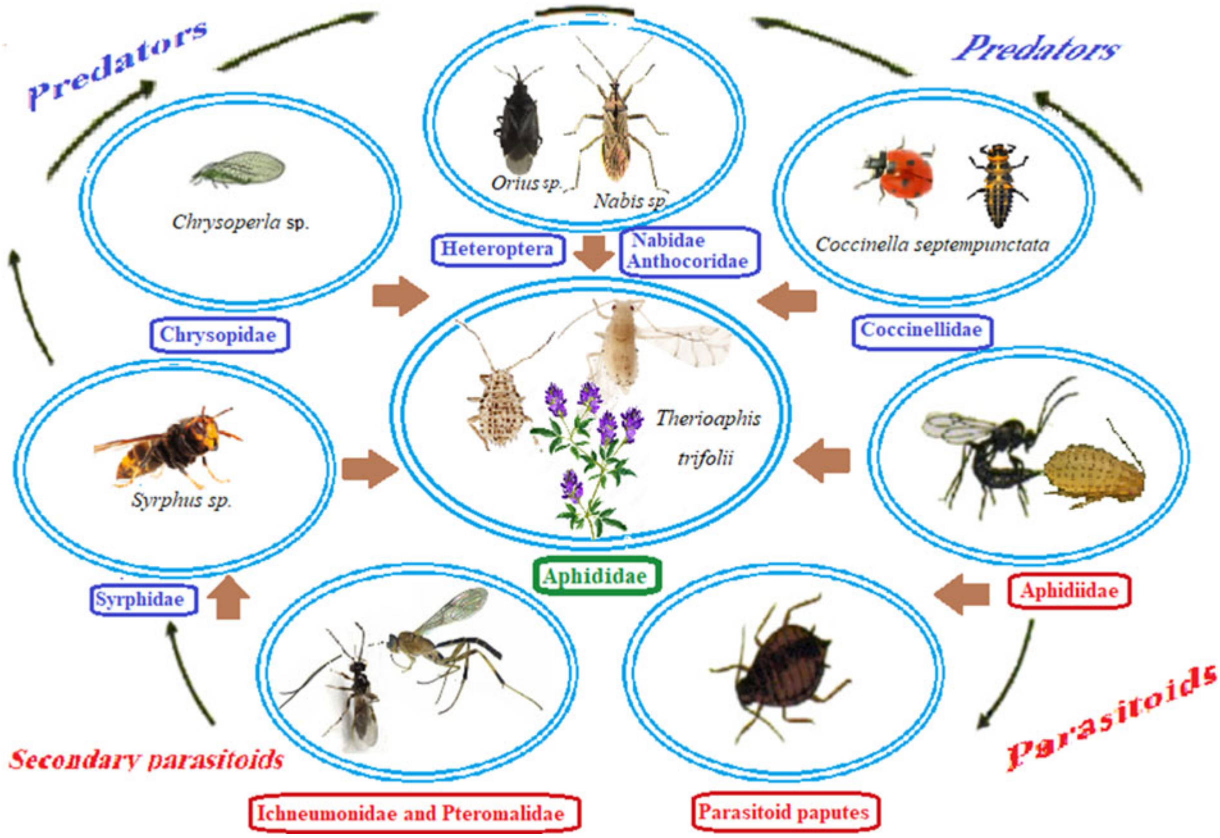
Family (order, suborder)	Diversity indices				
	Shannon diversity index (*H*)	Simpson's dominance index	Evenness	Margalef's species richness index	Effective number of species
		2015			
Aphididae (Sternorrhyncha, Hemiptera)	0.471	1.27	0.340	0.33	1.386
Coccinellidae (Coleoptera)	1.310	2.79	0.628	0.77	2.480
Anthocoridae, Nabidae (Heteroptera, Hemiptera)	0.977	2.09	0.705	0.33	2.000
Chrysopidae (Neuroptera)	0.532	1.40	0.485	0.22	1.446
Syrphidae (Diptera)	0.443	1.28	0.404	0.22	1.360
		2016			
Aphididae (Sternorrhyncha, Hemiptera)	0.790	1.71	0.491	0.44	1.729
Coccinellidae (Coleoptera)	1.430	2.75	0.687	0.76	2.700
Anthocoridae, Nabidae (Heteroptera, Hemiptera)	1.120	2.51	0.808	0.33	2.174
Chrysopidae (Neuroptera)	0.820	1.91	0.746	0.22	1.765
Syrphidae (Diptera)	0.605	1.48	0.55	0.22	1.521
		2017			
Aphididae (Sternorrhyncha, Hemiptera)	0.860	2.13	0.620	0.37	1.815
Coccinellidae (Coleoptera)	1.650	4.06	0.849	0.87	3.138
Anthocoridae, Nabidae (Heteroptera, Hemiptera)	0.717	1.55	0.517	0.37	1.644
Chrysopidae (Neuroptera)	0.778	1.87	0.708	0.25	1.715
Syrphidae (Diptera)	0.477	1.44	0.668	0.25	1.392
		2018			
Aphididae (Sternorrhyncha, Hemiptera)	0.224	1.10	0.162	0.36	1.168
Coccinellidae (Coleoptera)	1.770	4.98	0.849	0.83	3.411
Anthocoridae, Nabidae (Heteroptera, Hemiptera)	0.764	1.62	0.551	0.36	1.698
Chrysopidae (Neuroptera)	0.600	1.53	0.547	0.24	1.516
Syrphidae (Diptera)	0.212	1.12	0.306	0.24	1.158

The first adult syrphid fly (Syrphidae) was observed at the end of April (fig. 4). Favourable weather conditions in early May led to a higher abundance of syrphid flies. Intense migration commenced in the latter half of May, with densities peaking in mid-June. The concurrent mass reproduction of *T. trifolii* and *A. pisum* provided sufficient food for the predatory larvae of the Syrphidae family, resulting in a synchrony between predator and prey numbers. After oviposition, female syrphids died, leading to a significant reduction in the population. The development cycle of the flies spanned 3–4 weeks, with a new generation establishing itself in early July. A second peak was observed in mid-July, coinciding with a substantial decrease in prey availability. Syrphidae presence dwindled to a minimal level in August, with only single individuals detected in September.

The predatory ladybird population (Coccinellidae) began to increase in May. The first peak of the predominant species (*C. septempunctata* and *H. variegata*) occurred from the end of the month to the first half of June (fig. 5), corresponding to the abundance of their prey. Subsequently, ladybird numbers declined markedly, with minimal presence until the end of July, followed by a relative increase in August. The second period of high numbers was from the end of August, mainly in the first half of September, corresponding to the dynamic of aphid populations. Less abundant species, such as *Scymnus frontalis quadrimaculatus*, *Propylaea quatuordecimpunctata* and *C. quinquepunctata*, exhibited population dynamics similar to the dominant species. Overall, the variability in the total number of Coccinellidae species during the alfalfa growing season was less pronounced than in other aphidophagous groups.

Adult Chrysopidae individuals were observed in May (fig. 6) and were represented only by *Chrysoperla carnea*. Larvae appeared in June and July, as their number gradually decreased in August. The population of *C. carnea* increased in July and August, with individuals departing the crops by the end of August. *Chrysopa rhyllochroma*, present in June and July, and *C. commata*, observed in August, showed no significant variation in their numbers. Both *Chrysopa* species were additional components of the alfalfa insect community.

Aphidophagous predators played a decisive role in controlling aphid populations. It was crucial to evaluate the impact of predators, individually and collectively, on aphid responses. Regression analysis results (table 7) indicated that predator interactions significantly affected aphid population density. The coccinellid species exhibited the highest regression coefficient ($r = 138.79$),



Picture 1. Trophic interactions between the dominant aphid and its predators and parasitoids.

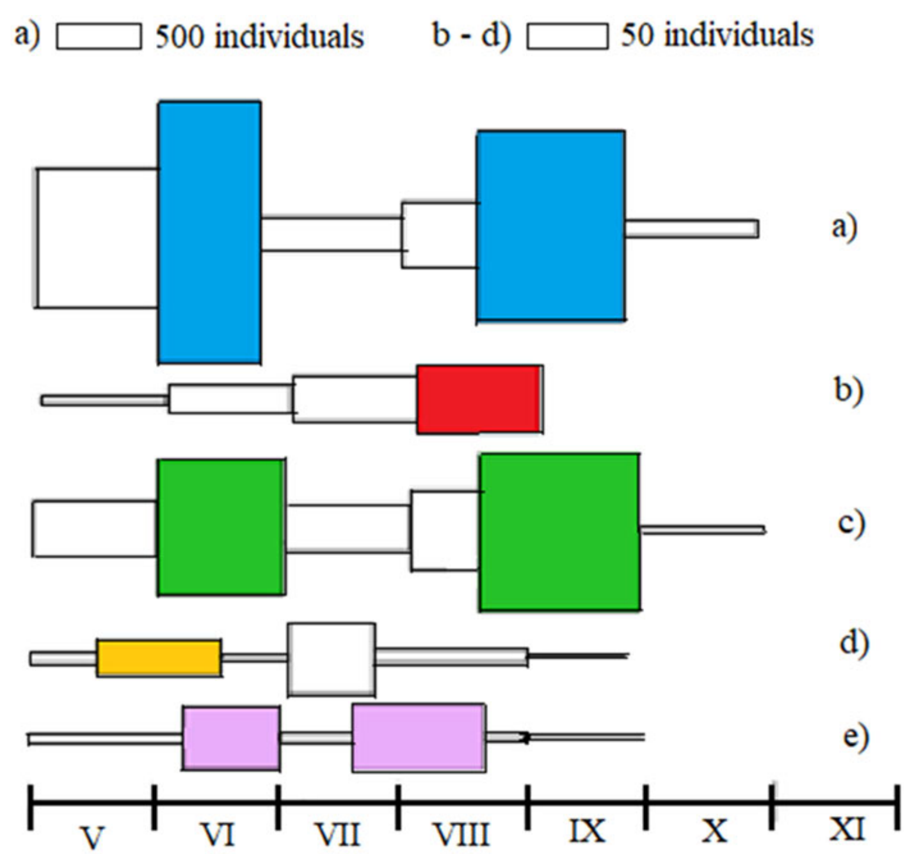


Figure 3. Dynamics of predators and aphids: (a) *Therioaphis trifolii* Monell and *Asurthosiphon pisum* Harris; (b) Chrysopidae; (c) Coccinellidae; (d) Syrphidae; (f) Nabidae – genus *Nabis* and Anthocoridae – genus *Orius.*

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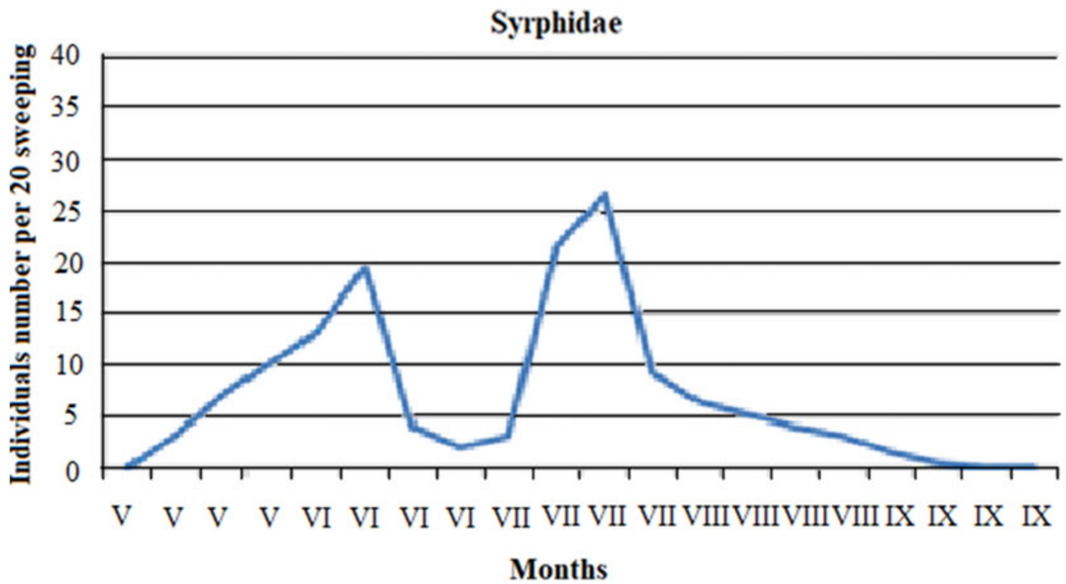

Figure 4. Population dynamics of the Syrphidae family (total number of adults and larvae).

strongly influencing aphid density. Ladybirds had a significant positive impact, whereas other aphidophagous predators had minimal and statistically insignificant effects on the complex interactions between predators and aphid populations.

From the order Hymenoptera, parasitoids from the Aphidiidae family, parasitising aphids, and from the Ichneumonidae and Pteromalidae families, which parasitise flies from the Syrphidae family, were identified.

Parasitoids from the Aphidiidae (primarily *Aphidius ervi* Haliday and to a lesser extent *Praon barbatum* Mackauer) and Aphelinidae (*Aphelinus* sp.) families were most numerous in 2016, followed by 2015 ($F_{3,7} = 2.491$; $P = 0.027$; $F_{3,7} = 1.631$; $P = 0.001$, respectively, table 2). The family of Ichneumonidae and Pteromalidae parasitising species from Syrphidae had the highest participation in 2016 followed by 2017 ($F_{3,7} = 1.883$; $P = 0.025$; $F_{3,7} = 2.174$; $P = 0.017$, respectively), with observed synchronisation in population dynamics between parasitoids and syrphids.

Discussion

The study examined the community structure, relationships and diversity among aphids and their aphidophagous species during 4 years. In the spring, as the weather warms, aphid larvae hatch and suck sap from the plant stems and leaves. Notably, the highest population densities for the two dominant aphid species, *T. trifolii*, and *A. pisum*, were observed during the second and fourth regrowth periods, respectively. The aphid abundance was closely synchronised with the population dynamics of their aphidophagous species. For example, the population of aphid-eating ladybirds was closely tied to the availability of their aphid prey, and

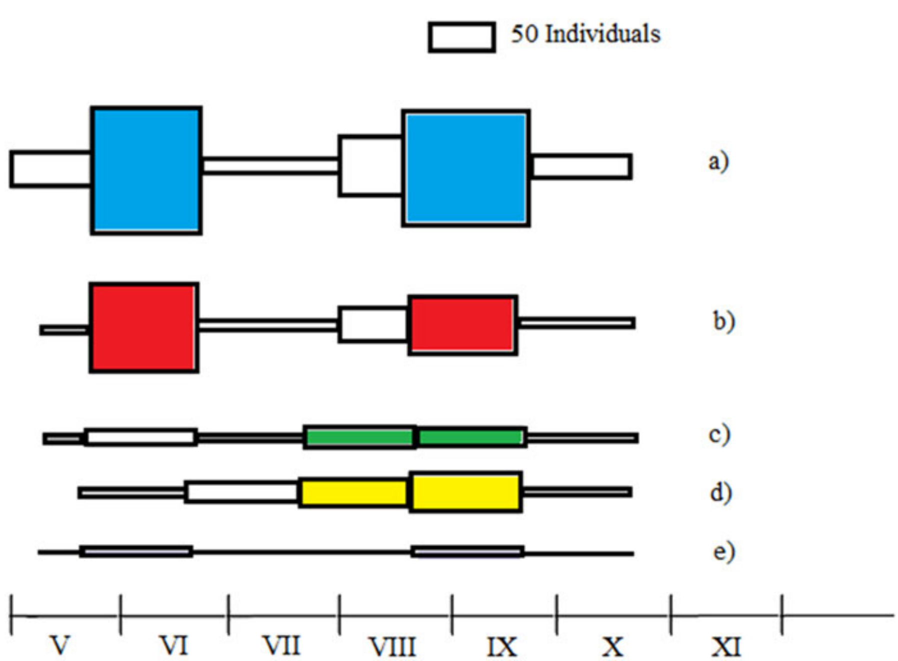

Figure 5. Dynamics of dominant species of the Coccinellidae family in alfalfa: (a) *Coccinella septempunctata* (adults and larvae); (b) *Hippodamia variegata*; (c) *Scymnus frontalis quadrimaculatus*; (d) *Propylaea quatuordecimpunctata*; (f) *Coccinella quinquepunctata* from (b) to (f) – adult individuals.

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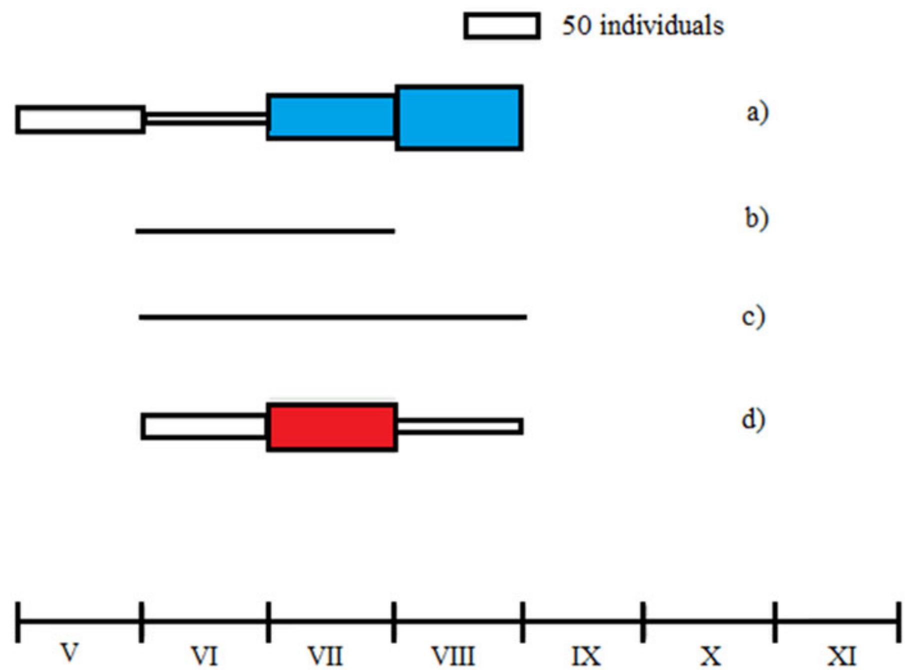

Figure 6. Dynamics of adults and larvae of the family Chrysopidae: (a) *Chrysoperla carnea* Stephens; (b) *Chrysopa phyllochroma* Wesmael; (c) *Chrysopa commata* Kis and Ujhelyi; (d) larvae.

the aphid abundance impacted ladybird abundance (fig. 3). Additionally, interactions found in the community of aphid–aphidophagous predatory bugs showed that ladybirds had a stable response and strongly influenced aphid density.

As the aphid numbers increased, so did the abundance of ladybirds, indicating a stable, density-dependent response of ladybirds to aphid populations, as also corroborated by previous research (Soleimani and Madadi, 2015; Arshad *et al*., 2017; Bálint *et al*., 2018; Pan *et al*., 2020; Ramandeep and Reddy, 2020).

The synchronous emergence and population fluctuations of aphids and their predators likely enhance the efficacy of biological control by these natural enemies. The timing of reproduction in aphidophagous species appears to be aligned with the generational overlap of aphid populations, facilitating continuous predator–prey interactions. This supports the findings by Soleimani and Madadi (2015) and Rajendra and Singh (2016), who highlighted the significant regulatory potential of aphid predators in agricultural fields. The complex trophic interactions between host plants, aphids and their natural enemies can significantly influence the success of biological control strategies (Jovičić *et al*., 2016; Shevchuk and Shevchuk, 2022).

Understanding the functional structure of species communities that depends on food resources is challenging but crucial. Bańkowska *et al*. (1975) emphasised the need to consider the ecological relationships within species, suggesting that the analysis of environmental systems could be based on food chain dynamics and the broader concept of food webs or competitive systems.

The alfalfa contained overlapping structural networks, involving trophic, competitive and paratrophic interactions. Predator groups included several ladybird species, predatory bugs, lacewings and syrphid flies. Findings indicated that aphids were the most significantly impacted by ladybird predators, particularly by dominant species such as *C. septempunctata* and *H. variegata* (table 7). The temperature and relative humidity were identified as the primary environmental factors influencing aphid and predator populations, with rainfall having a secondary and negative effect.

Table 7. Regression analysis and regression coefficients of the aphid density regarding their aphidophagous species

ANOVA	Degrees of freedom	Sum of squares	Mean squares	*F*	Significance *F*	
Regression	4	76,212,500.000	19,053,100.0	28.760	0.0002	
Residual	7	4,637,850.000	662,550.0			
Total	11	80,850,400.000				
			Regression coefficients			
Factors	Coefficients	Standard errors	*t* Statistic	*P*-value	Lower 95%	Upper 95%
Intercept	−549.700	395.385	−1.390	0.001	−1484.64	385.239
Coccinellidae	138.793	41.356	3.356	0.001	41.001	236.585
Chrysopidae	4.363	6.427	0.679	0.056	−10.834	19.559
Syrphidae	3.938	6.352	0.620	0.475	−11.083	18.958
Heteroptera	4.396	14.579	0.302	0.772	−30.078	38.869

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These results were consistent with those of Rakhshan *et al.* (2009) and Elliott *et al.* (2002), who also reported a high positive impact of the temperature on the insect population and direct numerical response by predators to aphid populations in alfalfa fields. The diversity and abundance of insects within the aphid–aphidophagous community were analysed, revealing that the Coccinellidae species exhibited the highest diversity, dominance and species richness among the insect groups studied. The pronounced dominance of Coccinellidae likely contributed to the high Shannon diversity index observed. The evenness values approached 1 in 2017 and 2018 (0.849 and 0.849, respectively), suggesting a more balanced community composition than in 2015 and 2016. In 2016, it was particularly notable for its high diversity and dominance indexes, as well as evenness and species richness among predator bugs, lacewings and syrphid flies. That emphasised the consistent influence of environmental factors on insect populations, as discussed by Okeke *et al.* (2019).

Biocontrol agents such as coccinellid, syrphid, bugs and chrysopid species are commonly found in alfalfa fields and are well-documented in various studies. In Bulgaria, Ivanova (2004) found in Plovdiv, Bulgaria that the useful heteropteran entomofauna was represented by two families – Nabidae and Anthocoridae, with *N. ferus* (Nabidae) being the most abundant. The *Anthocoris nemorum* L., *A. nemoralis* L. and *O. minutus* L. species (Anthocoridae) were less common in the alfalfa agrocenosis. In a comparative study, Atanasova (2006) reported on the predatory species of the genera *Nabis*, *Orius*, *Deraeocoris* and *Geocoris*. The density of heteropteran predators in alfalfa and their role in biological control were not accurately assessed according to Strawiński's (1964) report.

In contrast, in a study by Pons *et al.* (2009), Heteroptera was the most abundant and numerous suborder of predatory insects in alfalfa, with the most common species belonging to the families Nabidae, Anthocoridae and Miridae. Predatory bugs such as *Nabis* spp. accounted for 40–89% of predatory insects in Argentina (Cornelis *et al.*, 2012).

Similar results were reported by Razmjoo (2012), where the dominant species of the genera *Deracoris*, *Nabis*, *Orius* and *Geocoris* accounted for 60% of the total individuals collected in central Iran.

Furthermore, Razmjoo (2012) reported that the dominant species of the genera *Deracoris*, *Nabis*, *Orius* and *Geocoris* accounted for 60% of the total individuals collected in central Iran. Anthocoridae were also common alfalfa predators (Blodgett, 2009). The predominant species of this family were *O. niger*, *O. minutus*, *O. majusculus* and *Anthocoris confosus* (Bosco and Tavella, 2013). *Orius minutus* and *O. niger* were extremely effective predators against various herbivorous mites, insect eggs, aphids, thrips and small caterpillars due to their rapid development through all stages (Pons *et al.*, 2009; Konjević and Kereši, 2014).

In addition, the genus *Orius* played a crucial role in controlling phytophagous thrips populations worldwide. Bosco and Tavella (2013) reported that alfalfa-dominated *Orius* are known to be important bioagents against harmful thrips in Europe and the Middle East. According to the authors, *Orius* played the most important role in the thrips' biological control, and they had proven themselves, as well adapted to climatic conditions.

The ladybird species *C. septempunctata* and *H. variegata* can prey not only on various aphid species but also on plant bugs, psyllids, mites, cicadas and larvae of Chrysomelidae (Shevchuk and Shevchuk, 2022). Family Coccinellidae dominated, whose numbers accounted for more than two-thirds of the total number of this trophic level. The presence of local aphidophagous species could indicate that they can reduce the outbreak of aphid populations. Their possible effects (prey preference, voraciousness, etc.) on aphid species are the subject of further challenging studies. Similar results for the species composition of hard-winged aphids were reported by Jovičić *et al.* (2022) in alfalfa crops in Serbia. According to the authors, aphids *A. craccivora*, *A. kondoi* Shinji, *A. pisum* and *T. trifolii* were particularly important among the main phytophagous insects. Although aphid densities did not reach economically significant levels due to the presence of predatory insects, 13 species were observed to be significantly aphidophagous, including *C. septempunctata*, *H. variegata*, *P. quatuordecimpunctata*, *T. vigintiduopunctata, S. flavicollis* Redtenbacher (Coleoptera: Coccinellidae), *N. ferus* L. (Hemiptera: Heteroptera, Nabidae), *A. confusus* Reut (Hemiptera: Heteroptera, Anthocoridae), *C. carnea* Stephens (Neuroptera: Chrysopidae) and others (Jovičić *et al.*, 2016; El-Ghie, 2019).

Furthermore, the most common predatory species found in the study of Meseguer *et al.* (2021) were seven-spotted ladybirds and *H. variegata*, which positively correlated with pea aphid and *T. trifolii*, similar to the present experiment.

The family Syrphidae was less numerous than the Coccinellidae. The key oviposition role of syrphid flies was aphid colonies to provide food for their larvae. As the number of aphids increased, the predatory fly number also rose. Syrphid larvae have an advantage over other aphid-eating insects because they are slow and very greedy. That helped them effectively reduce the aphid population.

Syrphid flies had a high biocontrol potential against aphids (Haenke *et al.*, 2009). Rassoulian (2005) found that *S. cinctus* and *S. grassulariae* predominated among the predators of *A. pisum*, *T. trifolii* and *A. kondoi* in the alfalfa crop. Dominant species were also *E. corollae*, *C. septempunctata* and *H. variegata*; *C. carnea* and *N. capsiformis*. In the author's opinion, predators had the most significant impact on aphid population fluctuations. On the other hand, Nakashima and Akashi (2005) reported that the predominant predatory syrphid flies (Syrphidae) in alfalfa were *E. corollae* Fabricius, *Episyrphus balteatus* de Geer, *Metasyrphus ferquens* Matsumura and *S. vitripennis* Meigen.

As mentioned, the dominant chrysopid species in the study was *C. carnea*. Similarly, El-Ghie (2019) identified *C. carnea* as a common predatory species on aphids in alfalfa. Giles *et al.* (2000) investigated the feeding interactions between *A. pisum* and *Chrysoperla rufilabris* Burmeister in an alfalfa and found a high efficiency of these relationships.

The trophic structure of the food chain between aphids and parasitoids of the order Hymenoptera consisted of two links. The first link comprised specialised endoparasitoids that were difficult to identify due to two factors. The first challenge was the small body size of individuals in this group, leading to taxonomic research delays. The second challenge was the difficulty in collecting data on parasitism. During the study period, main parasitoids from the family Aphidiidae (and less from the family Aphelinidae) were found as parasitoids on aphids, and they showed the greatest parasitoid activity in 2016 due to their large numbers.

Parasitised aphids were characterised by their rather oval shape and light colour.

Similar results for increased parasitism of *Aphidius* parasitoid females with an increase in aphid density were reported by Khatri *et al.* (2021). Augmentation of natural enemies is the most used approach to biological control (Khatri *et al.*, 2021).

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However, the results for this group are incomplete and require further studies.

The second link – the chain of oligophagous parasitoids – contained parasitoid species from the order Hymenoptera, which specialised in parasitising aphids. Among the parasitoids were individuals from the families Ichneumonidae and Pteromalidae, which parasitised flies from the Syrphidae family mainly in 2016 and 2017.

The parasitoids were determined down to the family level, with 15 parasitised Syrphidae flies observed over the 4 years.

Aphids and their aphidophagous species were typical components in all terrestrial ecosystems according to Tomanović and Žikić (2018). The stability of community structure may refer to the overall structure of the community and the structure of its particular links. However, the species composition and number of individuals within particular relationships can also reverberate the stability of the specific association components within only one crop.

Order Hymenoptera is one of the most species-rich and abundant insect groups, with a remarkable range of life cycles, and over half of the Hymenoptera species have a parasitoid lifestyle (Forbes *et al.*, 2018). Farmers can gain much economically from using them as pest control agents.

Two independent factors were identified as having specific relationships. The first was that alfalfa is a perennial plant, so there was a succession of species during the 4 years, which showed the enrichment of the species composition. The second factor, associated with the species majority in the aphid–aphidophagous community, occurred in many ecosystems. Each community of species formed in the alfalfa was subject to relationships existing in a given part of the landscape.

In the study, syrphid flies emerged around mid-May. In the Plovdiv region, Bulgaria during a similar study, Ivanova (2004) found three species of syrphids that appeared relatively early – in the first 10 days of April, reaching their maximum numbers in May and June. Nakashima and Akashi (2005) reported that in alfalfa crops, syrphid predators synchronised population dynamics with those of *A. pisum* and *A. kondoi*. In addition, the authors found two species of parasitoids, *A. ervi* and *P. barbatum* (Aphidiine), on aphids, and their dynamics varied during the growing season. The approximate number of parasitised aphids reached its maximum parallel to the highest aphid number.

Ivanova (2004) found that Chrysopidae species were at their highest in alfalfa crops in the Plovdiv region in August and September, while in the Pleven region, Bulgaria syrphid flies reached two peak values – in mid-June and mid-July.

The structure of the aphid–aphidophagous community demonstrated that aphid population control could be highly efficient when changes in aphidophagous numbers are synchronised with aphid population dynamics. From mid-May to June, there was a substantial potential for aphidophagous predators to control aphids, while in September, predatory ladybirds of the Coccinellidae family played a pivotal role. The behaviour and seasonal ecology of parasitoid Hymenoptera species are needed to enrich the understanding of these dynamics.

Conclusions

Research on the structure of the aphid–aphidophagous community has identified three distinct groups: (1) polyphagous predatory bugs from the families Anthocoridae and Nabidae, (2) oligophagous and polyphagous predators from Coccinellidae, Syrphidae and Chrysopidae; and (3) monophagous and oligophagous parasitoids, predominantly from the families Aphidiidae and Ichneumonidae.

From mid-May to June, there was sufficient potential for aphidophagous species (Coccinellidae, Syrphidae, Chrysopidae, Anthocoridae and Nabidae) against aphids, while in September the predatory ladybirds of Coccinellidae were the main biological control agents. Coccinellidae (Coleoptera) exhibited the highest diversity, dominance index and species richness among the insect groups within the aphid–aphidophagous community. The existence of diverse aphidophagous species in alfalfa fields suggests that these predators can complement each other, leading to effective biological control of aphids. The synergy among different predator species holds promise for enhancing the overall efficacy of integrated pest management strategies.

Data availability. Data will be made available on request.

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