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Effect of Different Cover Crops, Mass-Trapping Systems and Environmental Factors on Invertebrate Activity in Table Olive Orchards—Results from Field Experiments in Crete, Greece

Nikolaos Volakakis [1,2,3,*], Emmanouil Kabourakis [1], Leonidas Rempelos [3,4], Apostolos Kiritsakis [5] and Carlo Leifert [6,7,*]

[1] Olive and Agroecological Production Systems Lab (EOPS), Hellenic Mediterranean University, 71410 Iraklio, Greece
[2] Geokomi Plc, Sivas Festos, P.O. Box 21, 70200 Crete, Greece
[3] Nafferton Ecological Farming Group, Newcastle University, Newcastle upon Tyne NE1 7RU, UK
[4] Lincoln Institute for Agri-Food Technology, University of Lincoln, Riseholme Park, Lincoln LN2 2LG, UK
[5] International Observatory of Oxidative stress for Health and Agricultural Products, 21 Kar. Diehl Str., 54623 Thessaloniki, Greece
[6] SCU Plant Science, Southern Cross University, Military Rd., Lismore, NSW 2480, Australia
[7] Department of Nutrition, IMB, University of Oslo, 0372 Oslo, Norway
* Correspondence: nvolakakis@gmail.com (N.V.); carlo.leifert@gmail.com (C.L.)

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Abstract: *Background:* Although the negative effects of insecticides and herbicides on beneficial and non-target invertebrates are well documented, there is limited information on potential negative impacts of pest and weed management practices used in organic farming on invertebrate activity. *Methods:* Using established field experiments designed to compare different ground cover crops (used to suppress weeds and increase nitrogen availability and soil health) and mass-trapping systems (used for olive fly control) in organic olive production systems, we monitored the impact of these practices on invertebrate activity. *Results:* When different ground cover crops were compared, ground cover crops established from a vetch/pea/barley seed mixtures resulted in significantly higher parasitic wasps activity than ground cover vegetation in control plots (plots in which *Medicago* seed were sown and failed to establish) that were dominated by the weed *Oxalis pes-caprae*. When two bottle based mass-trapping systems were compared, the traps caught similar numbers of olive flies and some non-target invertebrates (mainly other Diptera, Neuroptera and Lepidotera and Formicidae), although no parasitic wasps or pollinators (bees; bumble bees) were caught in traps. Analyses of invertebrate profiles found in McPhail monitoring traps showed that invertebrate activity profiles were similar in plots with and without mass-trapping devices. In addition, as expected, redundancy analyses showed that climatic parameters (temperature, rainfall, humidity, wind direction) are significant explanatory variables/drivers for invertebrate activity in olive orchards. *Conclusions:* The results presented indicate that mixed legume/cereal ground cover crops may increase the activity of parasitic wasps and may act as a reservoir for natural enemies of agricultural pest and that olive fly mass-trapping systems may lure and kill some non-target invertebrates, but do not affect the activity of two main groups of beneficial invertebrates namely pollinators and parasitic wasps.

Keywords: organic table olive production; ground cover crops; olive fly; mass-trapping; invertebrate activity; parasitic wasps; pollinators; invertebrate activity; *Oxalis pes-caprae*

1. Introduction

In the Mediterranean region, the olive fly (*Bactrocera oleae*, Rossi, Diptera: Tephritidae) is considered to be the economically most important pest in both table and olive oil production with yield losses of up to 80% having been reported [1–4]. In conventional olive production, the olive fly is controlled primarily by regular bait or cover spray application of insecticides [1,2]. The repeated use of pesticides has been shown to result in development or resistance in *Bactrocera oleae* against some of the most extensively used pyrethroid and organophosphorus insecticides and more recently also Spinosad, an insecticide which is permitted for use in organic production [2–4].

Another major challenge in olive production is ground vegetation management/weed control, which is not only important to (i) reduce competition for water and nutrients, but also to (ii) minimise wild fire risk in summer, (iii) improve soil health and fertility and (iv) increase the speed/efficiency and thereby reduce labour costs of the olive fruit harvest in autumn [5]. Ground cover management protocols may include (a) different types of tillage and/or hoeing, (b) mowing weeds/ground cover vegetation, (c) the use of grazing animals to remove ground cover vegetation and/or (d) the sowing of ground cover crops at the beginning of the rainy season in autumn [5–8]. There is a need to reduce tillage and mechanical weed control, because it contributes significantly to the fuel cost and carbon footprint of olive production [4]. In conventional olive production, herbicides are increasingly used for weed control, although herbicides such as glyphosate were reported to reduce olive yields [8].

There is increasing concern about the use of pesticides in olive production, since it is well documented to result in pesticide residues in both olive fruit and oil [9,10] and can have negative effects on non-target and potentially beneficial invertebrates including pollinators and natural enemies of invertebrate pests [11–16]. Negative impacts of pesticides (insecticides, herbicides, fungicides) have been documented for a wide range of beneficial invertebrates, including the parasitoid *Psyttalia concolor* Szépligeti (synomym *Opius concolor*, Hymenoptera, Braconidae) which is a natural enemy/parasite of *B. oleae* [16].

Organic farming systems prohibit the use of all synthetic chemical pesticides, although some insecticides made from plant extracts (e.g., pyrethrum) or microbial fermentation (Spinosad) are permitted [17]. However, although Spinosad is permitted, it is not thought to be widely used in organic production, due to its relatively high cost and the availability of efficient mass-trapping systems [1,18,19]. These baited traps, which contain food, colour and/or pheromone attractants and a lethal agent (e.g., an insecticide), are placed throughout an orchard prior to fruit setting and have been shown to be as effective as pesticide sprays in reducing fly numbers and fruit infestation, especially in Mediterranean regions with relatively low olive fly pest pressure [20–22].

Due to the contrasting cover management/weed control protocols used in organic and conventional olive production, the botanical composition, plant density and canopy structure differ considerably between the two systems, with herbicide use in conventional systems and the establishment of legume-based cover crops in organic systems being major explanatory variables [7,23].

The use of (i) mass-trapping systems (instead of insecticide sprays) for olive fly control and (ii) legume winter cover crops (instead of mineral N-fertilisers and herbicides) to increase soil N-levels and to suppress problematic weeds such as *Oxalis pes-caprae* L. are therefore considered the most important differences between organic and conventional olive production protocols that may affect invertebrate activity [7,18,19]. However, while the effects of synthetic chemical pesticides on non-target and beneficial invertebrates have been studied extensively [24–26], there is very limited information on the effects of olive fly mass-trapping systems and the use of legume winter cover crops on invertebrate activity in olive orchards.

Biodiversity benefits of organic farming have been reported for a range of crop production systems, and consumer perceptions that organic production delivers biodiversity benefits are an important "quality" driver of demand for organic products [27,28].

The main objective of the study reported here was therefore to investigate the effect of different legume cover crops and olive fly mass-trapping systems on invertebrate activity profiles, including (i) parasitic wasps belonging to the family Braconidae which includes the species *Bactrocera oleae* (the main invertebrate pest of olives), (ii) pollinators (honeybees and bumblebees) and (iii) natural enemies of invertebrate pest (e.g., ladybirds, net-winged insects and parasitic wasps).

2. Materials and Methods

2.1. Experimental Orchard Used

The field experiments used for studying invertebrate activity were established within an experimental table olive orchard at the National Agricultural Research Foundation of Greece (NAGREF) with 900 Kalamon cv. and 388 Manzanila cv. trees. At the time of the experiment, the trees had a height of 3.5–4 m and were planted 6 m apart. The orchard is located 8 km east (latitude 35°3′27, 33″ N, longitude 24°56′18, 22″ E, 158 m O.D.) of the town of Moires in the Messara plain in southern Crete, Greece (Figure 1). The orchard was in a landscape dominated by commercial olive fields (>50% of agricultural land area) and areas with wild olive trees and abandoned orchards.

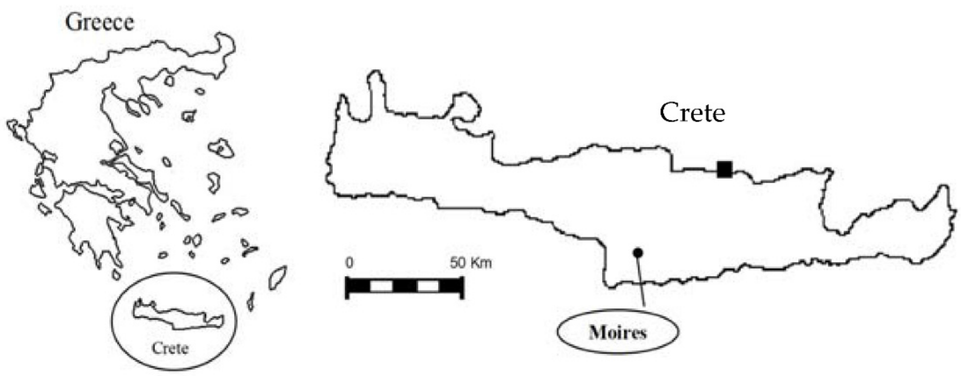

Figure 1. Location of experimental orchards in Crete, Greece.

2.2. Experimental Design of the Cover Crop Comparison Trial

A randomised block design was used incorporating four blocks of 48 Kalamon olive trees, each split into four treatment plots (12 trees/plot). In treatment plots, four different cover crop treatments were applied for three consecutive growing seasons (2005/2006, 2006/2007 and 2007/2008) to compare their effect of cover crops on (a) invertebrate activity (results reported in this article), and (b) *Oxalis* (*Oxalis pes-caprae* L.) establishment, olive yields, mineral supply to olive trees and olive fly infestation [7,18]. The four cover crop treatments were: (i) Vetch (*Vicia sativa* L.) without *Rhizobium* inoculation, (ii) a mixture of vetch (*Vicia sativa* L.), pea (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.), (iii) Vetch (*Vicia sativa* L.) with *Rhizobium* inoculation (Legume Fix, Legume Technology Ltd., Nottinghamshire, UK) and (iv) a native wild *Medicago* species (*Medicago polymorpha* L.). Since seeds of the native *Medicago* species showed very low establishment, the *Medicago* plots were used as a no cover crop control treatment [7]. The cover plot dimension/size is shown in Supplementary Figure S1, and climatic conditions (monthly rainfall and average mean daily temperature) during the three cover crop and olive growing seasons are summarised in Supplementary Figure S2. Details of the management of the cover crop plots during the experiment have been published previously [7,18].

2.3. Invertebrate Activity in Olive Tree Plots with Different Winter Ground Cover Crops

Invertebrate activity was determined in cropping periods 2006/2007 and 2007/2008 using the methods described by Stewart and Wright [29]. Three one-minute suction samples were taken from within the canopy of the cover crop vegetation and the olive trees canopy separately on sunny days in the first three weeks of April 2007 and 2008, after cover crops were fully established. Suction sampling was carried out using a modified Echo SHRED 'N' VAC PLUS ES-2400 leaf blower (Echo Inc., Lake Zurich, IL, USA) adapted as described in Stewart and Wright (1995). Suction samples within the cover crop vegetation canopy were taken at random in each plot (a total area of 432 m^2), avoiding edges by approximately four metres. Each olive tree canopy sample was taken immediately after the respective cover crop vegetation assessment, approximately 2 m above the soil surface, at the four cardinal points from the 2 middle trees of each plot (Figure 2).

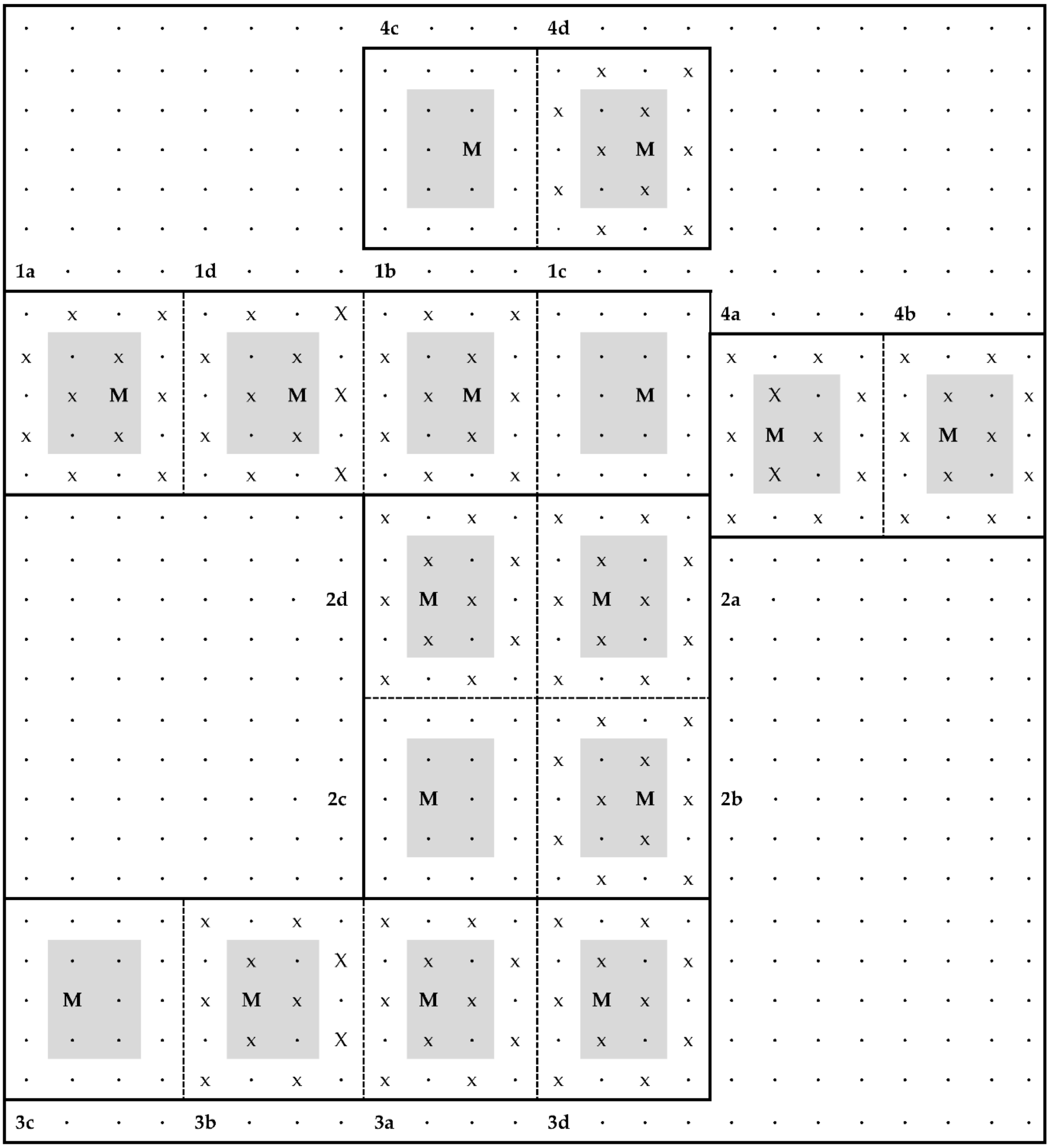

Figure 2. Experimental design of the olive fly mass-trapping system comparison trial within the experimental table olive orchard. Each small square has a 6 × 6 m dimension and an olive tree in the centre. Solid lines show the dimensions and positions of the 4 replicate blocks within the orchard. The four treatment plots within each replicate block are separated by dotted lines and labelled with lower case letter (**a**, Eco-Trap; **b,** Pepito trap; **c**, no trap control; **d**, Elkofon-1500 traps). An individual mass-trapping device was positioned in the canopy of every 2nd tree, and trees with a mass-trapping device are shown as x, while trees without a mass-trapping device are shown as λ; trees in which McPhail traps were placed to monitor olive fly activity are shown as **M**. The six trees in each treatment plot from which olive fruit samples for olive fly infestation assessment were collected are shown as grey shaded areas.

Invertebrate samples were kept in salt solution in the fridge and were later sorted/identified using a stereo-microscope [29].

2.4. Experimental Design of the Olive Fly Mass-Trapping System Comparison Trial

A randomized block design with four blocks of 80 Kalamon olive trees, each split into four treatment plots, was used (see Figure 2 for the arrangement of the different blocks and treatment plots within the olive orchard). At the time of the experiment, trees in each block were of similar height (3.5–4.0 m). In treatment plots, four different mass-trapping treatments were applied as part of an experiment focused on comparing their efficacy for olive fly control [19]. The four treatments, used in treatment plots, were: **(a)** control with no mass-trapping devices, **(b)** the Eco-Trap system (Vioryl S.A., Athens, Greece), 15 × 20 cm light green paper envelopes containing 70 g of ammonium bicarbonate salt, a food attractant and 15 mg of the insecticide deltamethrin on its surface, **(c)** Pepito traps, 1.5 L plastic bottles, with three holes drilled around the shoulder, containing 1 L of water with three torula yeast tablets (ISCA Technologies, Riverside, CA, USA), and **(d)** Elkofon-1500 traps, 1.5 L plastic trap with 0.5 L of the Entomela 55SL food attractant (Phytophyl S.A., Athens, Greece) and 1 L of water. The climatic background conditions in the three years (2006, 2007, 2008) in which mass-trapping systems were compared were the same as in the cover crop trial and are summarised in Supplementary Figure S2.

2.5. Monitoring of Environmental Background Conditions

Temperature and relative humidity were monitored hourly using a HOBO HO8 monitoring station (Onset Computer Corporation, Bourne, MA, USA) placed in a Stephenson screen in the middle of the orchard. Data were used to compute mean weekly and monthly temperature and humidity (Supplementary Figure S2).

Rainfall and wind strength and direction data for the period 2006–2008 were obtained from the weather station at the Messara Research Station, which is part of the Greek National Meteorological Service network and located 225 m from the survey orchard. Both rainfall and wind strength and direction were recorded 3 times per day at 06:00 a.m., 12:00 a.m. and 06:00 p.m. These data were used to calculate weekly total rainfall and a weekly wind direction index (frequency × strength) for the 4 main wind directions.

2.6. Olive Fly and Invertebrate Activity Monitoring

Olive fly activity was monitored using McPhail traps, which were based on the food attractant Entomela 55SL. McPhail traps are a standard method for assessing olive fly numbers [30] and are used widely in the Mediterranean [31,32]. Every year, traps were put out in the beginning of February, and samples were taken weekly until the middle of November, with a total of 120 samples being collected over the 3 years. A total of 16 McPhail traps were used, one in the centre of each treatment plot (Figure 2). The samples were taken back to the laboratory, where numbers of olive flies, Mediterranean fruit flies and other invertebrates were counted. McPhail traps were used from the end of February to the beginning of December in all 3 years.

2.7. Olive Fly Fruit Infestation Estimation

Estimates of olive fly fruit infestation levels were carried out by collecting olive fruit samples from six trees in the centre of each treatment plot per sampling date (Figure 2). A total of 120 fruit were collected form the six trees (20 per tree) and examined for active and non-active infestation, recording egg punctures, alive and dead eggs and larvae as infestation. Assessments were carried out every two weeks from the 1st of July until the 15th of November in the two harvest years (2006 and 2008).

2.8. Invertebrates Caught by Bottle-Based Mass-Trapping Systems

Assessments of invertebrates caught in the two bottle-based mass-trapping systems (Pepito or Elkofon) were carried out to quantify the number of olive flies and non-target invertebrates caught in the two different mass traps, in order to compare the impact of traps on both **(a)** olive fly populations and **(b)** non-target and potentially beneficial insects.

Assessments were carried out in September in all 3 years (2006, 2007 and 2008). A total of 4 traps per plot were used, and invertebrates found were identified as either olive flies (*Bactrocera oleae*), Mediterranean fruit fly (*Ceratitis capitata*), other Diptera, Neuroptera, Lepidoptera, Coleoptera, ants (Formicidae, Hymenoptera) or other Hymenoptera. In all three years, assessments were carried out 14 weeks after the establishment of mass traps, when traps had caught a substantial number of invertebrates, but before decomposition made identification too difficult.

The liquid containing invertebrates was removed from the bottle trap through a mesh sieve. The invertebrates collected in the sieve were then placed onto a white plastic tray with a raised rim and then separated by adding sterile distilled water. A sub-sample of approximately 50 (+/−20) individual insects was taken and was separated into groups belonging to different taxonomic insect groups. The numbers belonging to different groups were counted, and all invertebrates counted per trap were placed into a separate 5 mL tube.

2.9. Statistical Analyses

The effects of, and interactions between factors, on measured parameters were assessed by analysis of variance (ANOVA) derived from linear mixed-effects (LME) models [33] by using the "nlme" package in R [34]. The hierarchical nature of the design was reflected in the random error structures. The normality of the residuals of all models was tested using quantile–quantile (QQ) plots. Real means and standard errors of means were generated by using the "tapply" function in R.

The relationships between environmental factors and invertebrate activity (McPhail trap invertebrate count data) were investigated using partial redundancy analysis (pRDA), which summarizes the amount of invertebrate activity that is explained by the selected environmental variables, after removing the effects of other variables (block = replicate) and by using the CANOCO 5 software [35]. Automatic forward selection of the agronomic or phenolic factors within the RDAs was used and their significance in explaining additional variance calculated using Monte Carlo permutation tests.

3. Results

3.1. Effect of Cover Crops on Invertebrate Activity in the Cover Crop and Olive Tree Canopy

The effects of cover crop and sampling position on the activity of different invertebrate orders/families were studied by carrying out suction sampling in April (after cover crops had fully established) in two consecutive years (2007 and 2008) in two sampling positions (a) above the soil within the cover crop canopy and (b) within the olive tree canopy.

A significant main effect of cover crop was only detected for the three parasitic wasp families (Braconidae, Ichneumonidae, Pteromalidae) (Tables 1 and 2). Parasitic wasp activity was highest in plots with cover crops established with a vetch/pea/barley seed mixture (Tables 1 and 2), which was also shown to result in the lowest density (134 ± 8 plants/m^2) of Oxalis (*Oxalis pes-caprae*), the main weed species found in olive orchards in the Messara region [7,19]. Parasitic wasp activity was lowest in plots with cover crops established with seed of native *Medicago* legume species (Tables 1 and 2), which had a very low *Medicago* density (9 ± 1 plants/m^2) and a significantly higher Oxalis density (253 ± 11 plants/m^2) compared with the vetch (134 ± 6 plants/m^2) and Oxalis (134 ± 8 plants/m^2) densities recorded in the cover crops established with the vetch/pea/barley seed mixture [7,19].

Table 1. Effects of, and interactions between, **(a)** year (2007 or 2008), **(b)** cover crop treatment (vetch established with Rhizobium inoculated seed, vetch established with untreated seed, vetch/barley/pea mixture or Medicago), **(c)** sampling position (cover crop or olive tree canopy) on the activity (mean numbers of invertebrates collected by suction sampling) of major invertebrate groups/orders in table olive cv Kalamon orchards. Values shown are main effect means ± SE.

				Pseudo-Scorpiones		Hymenoptera	
Factor	**Diptera (Flies)**	**Hemiptera (True Bugs)**	**Araneae (Spiders)**	**(Pseudo-Scorpions)**	**Cole-optera (Beetles)**	**Formi-cidae (Ants)**	**Parasitic Wasp Families [1]**
Year							
2007 (*n* = 32)	39 ± 4	1.2 ± 0.3	2.2 ± 0.4	2.2 ± 0.6	9.9 ± 1.1	5.7 ± 1.3	7.5 ± 1.4
2008 (*n* = 32)	27 ± 3	17.4 ± 3.6	5.6 ± 1.2	0.1 ± 0.1	2.1 ± 0.6	2.8 ± 0.4	8.7 ± 1.2
Cover crop							
vetch +*Rhizobium* [1] (*n* = 16)	36 ± 8	13.8 ± 7.2	4.9 ± 1.9	1.3 ± 0.7	6.0 ± 1.6	5.0 ± 2.0	9.2 ± 2.1 **ab**
vetch -*Rhizobium* [2] (*n* = 16)	31 ± 4	8.4 ± 3.0	3.2 ± 1.0	0.8 ± 0.4	5.8 ± 1.4	4.0 ± 1.1	7.1 ± 1.3 **bc**
vetch/pea/barley [3] (*n* = 16)	39 ± 6	7.9 ± 2.4	3.8 ± 1.4	0.9 ± 0.7	6.3 ± 2.0	4.0 ± 1.2	12.0 ± 2.2 **a**
Medicago [4] (*n* = 16)	27 ± 3	7.1 ± 1.8	3.6 ± 0.7	1.5 ± 0.8	6.0 ± 1.5	3.9 ± 1.1	4.0 ± 1.0 **c**
Sampling position							
cover crop (*n* = 32)	44 ± 4	11.7 ± 3.9	6.4 ± 1.1	2.2 ± 0.6	6.7 ± 1.1	7.8 ± 1.0	12.0 ± 1.5
olive tree (*n* = 32)	23 ± 2	7.0 ± 1.3	1.4 ± 0.3	0.0 ± 0.0	5.4 ± 1.2	0.7 ± 0.1	4.1 ± 0.5
ANOVA results (*p*-value)							
Main effects							
Year (YR)	0.0076	0.0001	0.0009	0.0001	<0.0001	0.0027	NS
Cover crop (CC)	NS	NS	NS	NS	NS	NS	0.0023
Sampling position (SP)	<0.0001	NS	<0.0001	<0.0001	NS	<0.0001	<0.0001
Interactions							
YR × CC	NS	NS	NS	NS	NS	NS	NS
YR × SP	NS	NS	0.0003	0.0001	T	0.0009	NS
CC × SP	NS	NS	NS	NS	NS	NS	NS
YR × CC × SP	NS	NS	NS	NS	NS	NS	NS

NS, not significant ($p > 0.1$); T, trend ($0.1 > p > 0.05$); [1], see Table 2 for the activity of different Hymenoptera families that only or mostly include parasitic wasp species; [2], vetch established with *Rhizobium* inoculated seed; [3], vetch established with untreated seed; [4], cover crop established with a vetch, pea, barley seed mixture.

Significant main effects of sampling position (cover crop versus olive tree canopy) were detected for a wider range of invertebrate orders, namely Diptera (flies), Araneae (spiders), Pseudoscorpiones (pseudo-scorpions), and the Formicidae (ants) and three parasitic wasp families of the Hymenoptera monitored (Tables 1 and 2); activity for all orders/families listed was higher in the cover crop compared with the olive tree canopy (Tables 1 and 2).

Significant effects of year were detected for all invertebrate orders and the Formicidae (ants) family of the Hymenoptera (Table 1), but not for the three parasitic wasp families of the Hymenoptera and the individual families of the Diptera (flies) (Table 2). Total Diptera, Pseudoscorpiones and Formicidae activity was higher in 2007, while Hemiptera (true bugs) and Araneae (spiders) activity was higher in 2008 (Table 1).

ANOVA also detected significant interactions between (a) year and sampling position (cover crop versus olive tree canopy) for the activity of Araneae (spiders), Pseudoscorpiones (pseudo-scorpions), Formicidae (ants) and parasitic wasps belonging to the Braconidae and Ichneumonidae family of the Hymenoptera, and (b) cover crop and sampling position for the activity of parasitic wasps belonging to the Pteromalidae family of the Hymenoptera (Tables 1 and 2).

Table 2. Effect of, and interactions between, **(a)** year (2007 or 2008), **(b)** cover crop treatment (vetch established with Rhizobium inoculated seed, vetch established with untreated seed, vetch/barley/pea mixture or Medicago), **(c)** sampling position (cover crop or olive tree canopy) on the activity (mean numbers of invertebrates collected by suction sampling) of the different invertebrate families belonging to the order Coleoptera (beetles) and Hymenoptera [1] in table olive cv Kalamon orchards. Values shown are main effect means ± SE.

Factor	Coleoptera (Beetles)			Hymenoptera (Parasitoid Wasp Families)		
	Staphylinidae (Rove Beetles)	**Cantharidae (Soldier Beetles)**	**Coccinelidae (Ladybirds)**	**Braconidae**	**Ichneu -monidae**	**Ptero-malidae**
Year						
2007 (n = 32)	1.2 ± 0.4	1.9 ± 1.1	0.3 ± 0.1	3.0 ± 0.7	2.4 ± 0.4	1.3 ± 0.3
2008 (n = 32)	0.6 ± 0.3	3.3 ± 0.9	0.4 ± 0.1	3.3 ± 0.5	3.2 ± 0.6	2.0 ± 0.4
Cover crop						
vetch +*Rhizobium* [1] (n = 16)	0.5 ± 0.3	5.2 ± 2.7	0.5 ± 0.2	3.4 ± 0.8 **ab**	3.7 ± 1.0 **ab**	1.4 ± 0.7 **bc**
vetch -*Rhizobium* [2] (n = 16)	0.3 ± 0.2	2.9 ± 0.9	0.4 ± 0.2	2.8 ± 0.6 **ab**	2.3 ± 0.5 **ab**	1.8 ± 0.4 **ab**
vetch/pea/barley [3] (n = 16)	1.2 ± 0.6	1.4 ± 0.6	0.3 ± 0.1	4.8 ± 1.3 **a**	3.8 ± 0.9 **a**	2.9 ± 0.6 **a**
Medicago [4] (n = 16)	1.7 ± 0.6	0.8 ± 0.3	0.3 ± 0.1	1.8 ± 0.6 **b**	1.7 ± 0.5 **b**	0.3 ± 0.2 **c**
Sampling position						
cover crop (n = 32)	1.8 ± 0.4	3.7 ± 1.4	0.6 ± 0.1	4.9 ± 0.7	4.3 ± 0.6	2.2 ± 0.5
olive tree (n = 32)	0.0 ± 0.0	1.5 ± 0.4	0.1 ± 0.1	1.4 ± 0.2	1.3 ± 0.2	1.0 ± 0.2
ANOVA results (p-value)						
Main effects						
Year (YR)	NS	NS	NS	NS	NS	NS
Cover crop (CC)	T	NS	NS	0.0355	0.0369	0.0054
Sampling position (SP)	<0.0001	NS	0.0007	<0.0001	<0.0001	0.0149
Interactions						
YR × CC	NS	NS	NS	NS	NS	NS
YR × SP	NS	NS	NS	T	0.0067	NS
CC × SP	T	NS	NS	0.0399	NS	NS
YR × CC × SP	NS	NS	NS	NS	NS	NS

NS, not significant ($p > 0.1$); T, trend ($0.1 > p > 0.05$); means with the same lower case letter for the same factor within the same column are not significantly different according to Tukey's honest significant difference test (THSD; $p < 0.05$); [1], see Table 1 for results obtained for the Hymenoptera family Formicidae (ants); [2], vetch established with *Rhizobium* inoculated seed; [3], vetch established with untreated seed; [4], cover crop established with a vetch, pea, barley seed mixture.

When the interactions between year and sampling position were further investigated, (a) ant activity in the cover crop canopy was significantly higher in 2007 than 2008, while there was no significant difference in activity between years in the olive tree canopy, (b) spider activity in the cover crop canopy was significantly higher in 2008 than 2007, while there was no significant difference in activity between years in the olive tree canopy, (c) Pseudoscorpion activity was only detected in the cover crop canopy in 2007, (d) beetle activity was higher in 2007 than 2008 in both the cover crop and olive tree canopy, but a significant difference between sampling positions was only detected in 2008, (e) activity of the parasitic wasp family Braconidae was significantly higher in the cover crop than the olive tree canopy, but the relative difference between sampling positions was greater in 2007 than 2008, and (f) activity of the parasitic wasp family Ichneumonidae was significantly higher in the cover crop than the olive tree canopy, but the relative difference between sampling positions was greater in 2008 than 2007 (Figure 3).

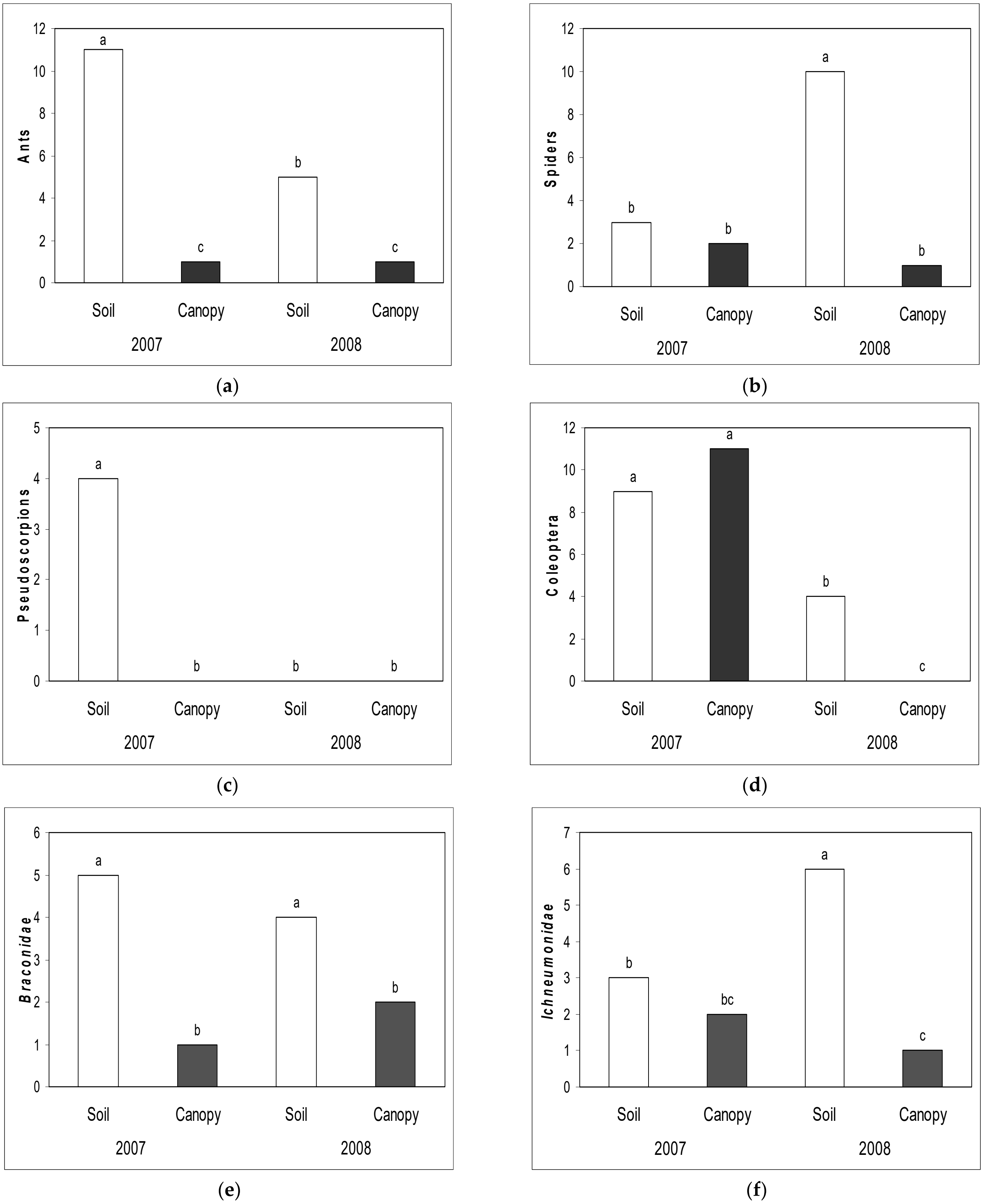

Figure 3. Effect of year and sampling position (soil = above the soil, within the cover crop canopy; canopy = within the olive tree canopy) on the activity of (**a**) Formicidae (ants), (**b**) Araneae (spiders), (**c**) Pseudoscorpiones (pseudoscorpions), (**d**) Coleoptera (beetles) and the parasitic wasp families (**e**) Braconidae and (**f**) Ichneumonidae. Values shown are interaction means ± SE (see Tables 1 and 2 for the *p* values of the year x sampling position interactions). Bars with the same letter within each figure are not significantly different according to THSD tests ($p < 0.05$).

When the interaction between cover crop and sampling position was investigated, differences in activity of parasitic wasps belonging to the Pteromalidae were found to be greater in the cover crop than the olive tree canopy (individual interaction means not shown).

3.2. Effect of Mass-Trapping Systems on Invertebrate Activity in Olive Orchards

The effect of mass-trapping devices on the activity of invertebrates belonging to different order/families was determined by two different approaches. Specifically (i) for the two plastic bottle mass-trapping systems (Pepito, Elkofon), we compared the numbers of olive flies and other invertebrates caught in traps in three consecutive years (2006, 2007 and 2008) and (ii) for all four treatments (no-trap control, Pepito, Elkofon and Vioryl), we assessed number of invertebrates belonging to different order/families that were found in McPhail monitoring traps in five 8-week periods between February and November in three consecutive years (2006, 2007 and 2008). We also assessed olive fly fruit infestation levels at monthly intervals between July and November in the two harvest years 2006 and 2008.

When invertebrates caught in the two bottle traps (Pepito, Elkofon) were compared, the number of olive flies caught in the two traps was similar (Table 3). However, significant main effects of the mass-trapping system were found for Diptera (flies) other than olive fly or Mediterranean fruit fly, Coleoptera (beetles) and Lepidoptera (butterflies and moths), with ~3-times higher other Diptera and Lepidoptera caught in Pepito traps and ~2.5-times higher Coleoptera caught in the Elkofon traps (Table 3).

Table 3. Effect of, and interactions between, **(a)** year (2007 or 2008), **(b)** plastic bottle-based mass-trapping system (Pepito or Elkofon) on the mean number of invertebrates belonging to different **orders** (common names) caught in bottle traps positioned in table olive cv Kalamon trees.

	Diptera (Flies)					**Lepidoptera**	**Hymenoptera** [1]
Factor	**Bactrocera oleae (Olive Fly)**	**Ceratitis capitata (MFF)**	**Other Diptera**	**Neuroptera (Net-winged Insects)**	**Coleoptera (Beetles)**	**(Butterflies and Moths)**	**Formicidae (Ants)**
Year							
2006	207 ± 31	0 ± 0	221 ± 46 **b**	21 ± 4	7 ± 2 **ab**	43 ± 14	9 ± 5 **b**
2007	166 ± 18	21 ± 5	711 ± 150 **a**	53 ± 14	5 ± 2 **b**	54 ± 12	104 ± 33 **a**
2008	102 ± 11	9 ± 3	163 ± 21 **b**	24 ± 3	11 ± 2 **a**	64 ± 12	16 ± 6 **b**
Trap type							
Pepito	162 ± 22	15 ± 4	575 ± 110	46 ± 10	4 ± 1	64 ± 12	70 ± 24
Elkofon	159 ± 16	15 ± 4	172 ± 29	20 ± 2	10 ± 2	19 ± 3	18 ± 4
ANOVA results (p-value)							
Main effects							
Year (YR)	NS	T	<0.001	NS	0.001	NS	<0.001
Trap type (TT)	NS	NS	<0.001	NS	<0.001	0.003	NS
Interaction (YR × TT)	NS	T	T	NS	0.013	NS	NS

MFF, Mediterranean fruit fly; NS, not significant ($p > 0.1$); T, trend ($0.1 > p > 0.05$); means with the same lower case letter for the same factor within the same column are not significantly different according to Tukey's honest significant difference test (THSD; $p < 0.05$); [1], see Table 2 for the activity of different Hymenoptera families that only or mostly include parasitic wasp species.

Significant numbers of Neuroptera (net-winged insects) and Formicidae (ants) and some Mediterranean fruit flies were also caught in the two traps, but no bees, bumble bees and insects belonging to the three parasitic wasp families (Braconidae, Ichneumonidae, Pteromalidae) of the Hymenoptera were found in the two different mass-trapping devices.

Significant main effects of year were detected for the activity of Diptera (flies) other than olive fly or Mediterranean fruit fly (highest in 2007 and lowest in 2008), Coleoptera (highest in 2008 and lowest in 2007) and Formicidae (highest in 2007 and lowest in 2006) (Table 3).

When olive fly fruit infestation was compared, two-factor ANOVA identified no significant main effect of trapping system, but a significant main effect of harvest year, with higher fruit infestation, was found to be higher in 2006 than 2008 (individual results not shown). In addition, when fruit infestation was compared in different sampling months in 2006 and 2008, no significant effect of mass-trapping treatment was identified (Table 4).

Table 4. Effect of mass-trapping system on olive fly (*Bactrocera oleae*) fruit infestation (% fruit infested) at different times during the growing season.

		Mass-Trapping System			
Harvest Year (Sampling Month)	**Control (No Trap)**	**Vioryl Green Plasticised Paper Envelope (Insecticide [1]; NH_4 Attractant)**	**Pepito 1.5 L Water Bottle (*Torula* Yeast Attractant)**	**Elkofon Commercial Bottle (NH_4 Attractant)**	**ANOVA Results (*p*-Values)**
2006					
July	2.3 ± 0.9	1.4 ± 0.6	1.5 ± 0.4	0.9 ± 0.3	NS
August	1.9 ± 0.4	3.6 ± 0.5	3.0 ± 0.2	2.3 ± 0.5	T
September	1.9 ± 1.1	1.9 ± 0.9	2.5 ± 1.0	1.3 ± 0.2	NS
October	3.8 ± 0.9	4.2 ± 0.7	3.6 ± 1.5	2.8 ± 1.1	NS
November	3.8 ± 1.1	4.0 ± 0.9	4.0 ± 1.6	3.3 ± 1.2	NS
Mean	2.7 ± 0.7	3.0 ± 0.4	2.9 ± 0.8	2.1 ± 0.3	NS
2008					
July	0.9 ± 0.3	0.6 ± 0.3	0.3 ± 0.3	0.4 ± 0.2	NS
August	0.7 ± 0.2	0.4 ± 0.2	0.4 ± 0.4	0.3 ± 0.1	NS
September	1.4 ± 0.4	0.5 ± 0.3	0.7 ± 0.4	0.7 ± 0.3	NS
October	1.5 ± 0.5	0.6 ± 0.3	1.2 ± 0.3	0.8 ± 0.2	NS
November	1.9 ± 0.9	2.1 ± 0.2	3.1 ± 1.1	1.5 ± 0.2	NS
Mean	1.3 ± 0.3	0.9 ± 0.2	1.2 ± 0.3	0.8 ± 0.1	NS

Values shown are means ± SE; NS, not significant ($p > 0.1$); T, trend ($0.1 > p > 0.05$); [1] the insecticide included in the trap was the synthetic pyrethroid deltamethrin.

When data from the McPhail monitoring traps were analysed, we carried out separate ANOVAs for (a) the first two 8-week sampling periods (mid-February to mid-April, mid-April to early-June) and (b) the last three 8-week sampling periods (early June to early August, early August to late September, late September to late November). This was performed because in the first two 8-week periods, no mass-trapping devices were present in the experimental plots. Mass-trapping devices were placed into experimental plots in early June and remained in trees during into the end of the last 8-week monitoring period in November.

As expected, ANOVA detected no significant main effects of mass-trapping system on invertebrate activity profiles in the first two 8-week monitoring periods, when there were no mass-traps in the experimental plots (Table 5).

In contrast, ANOVA detected a significant main effect of the mass-trapping system on olive fly activity, but not any of the other invertebrate orders/families found in McPhail traps. Specifically, olive fly activity was highest in Pepito plots and lowest in Vioryl plots, but olive fly activity was very low in all treatment plots and that Tukey's honest significant difference test did not detect significant differences between mass trap treatments, including the no trap control treatment (Table 6).

Table 5. Effect of, and interactions between, **(a)** year (2006, 2007, 2008), **(b)** 8-week period (mid-February to mid-April, mid-April to early-June), and **(c)** mass-trapping system on the activity (insects caught in McPhail monitoring traps) of major invertebrate groups/orders before mass-trapping systems were placed into table olive trees.

Factor	Bactrocera oleae (Olive Fly)	Lepidoptera (Butterflies and Moths)	Coleoptera (Beetles)	Hymenoptera (Parasitic Wasp Families [1])	Neuroptera (Net-Winged Insects)
Year					
2006	13.2 ± 1.1 **b**	7.4 ± 1.5 **a**	6.7 ± 2.1	7.0 ± 1.4	2.3 ± 0.5
2007	32.8 ± 2.3 **a**	6.9 ± 1.2 **a**	7.6 ± 2.2	10.8 ± 2.2	1.3 ± 0.3
2008	17.0 ± 1.8 **b**	2.6 ± 0.5 **b**	6.1 ± 1.7	5.3 ± 1.1	2.3 ± 0.5
8-week period					
mid Feb. to mid Apr.	18.7 ± 1.3	0.2 ± 0.1	0.2 ± 0.1	1.9 ± 0.5	0.2 ± 0.1
mid Apr. to early Jun	23.3 ± 2.3	11.1 ± 0.9	13.5 ± 1.9	13.5 ± 1.4	3.7 ± 0.3
Mass trap					
Control (no traps)	22.8 ± 2.9	4.7 ± 1.1	5.2 ± 1.8	5.5 ± 1.1	1.4 ± 0.3
Vioryl	21.5 ± 2.7	6.4 ± 1.6	9.0 ± 3.5	9.2 ± 2.9	2.3 ± 0.6
Pepito	19.6 ± 2.3	5.4 ± 1.4	6.0 ± 1.7	7.4 ± 1.4	1.8 ± 0.5
Elkofon	20.1 ± 2.9	6.1 ± 1.6	7.0 ± 1.9	8.6 ± 1.7	2.3 ± 0.6
ANOVA results (*p*-value)					
Main effects					
Year (YR)	0.0001	0.0068	NS	NS	T
8-week period (8WP)	0.0076	<0.0001	0.0001	<0.0001	<0.0001
Mass trap (MT)	NS	NS	NS	NS	T
Interactions					
YR × 8WP	<0.0001	0.0030	NS	NS	0.0058
YR × MT	NS	NS	NS	NS	NS
8WP × MT	NS	NS	NS	NS	NS
YR × 8WP × MT	NS	NS	NS	NS	NS

NS, not significant (>0.1); T, trend (0.1 > *p* > 0.05). [1], Braconidae, Ichneumonidae and Pteromalidae.

Table 6. Effect of, and interactions between, **(a)** year (2006, 2007, 2008), **(b)** 8-week period (early June to early August, early August to late September, late September to late November), and **(c)** mass-trapping system (on the activity (insects caught in McPhail monitoring traps) of major invertebrate groups/orders after mass-trapping systems were placed into table olive trees.

Factor	Bactrocera oleae (Olive Fly)	Lepidoptera (Butterflies and Moths)	Coleoptera (Beetles)	Hymenoptera (Parasitic Wasp Families [1])	Neuroptera (Net-Winged Insects)
Year					
2006	11.3 ± 2.2 **a**	6.7 ± 1.4 **a**	0.17 ± 0.04	0.59 ± 0.11	2.3 ± 0.4 **a**
2007	6.2 ± 0.6 **b**	1.6 ± 0.2 **b**	0.37 ± 0.11	0.68 ± 0.13	3.2 ± 0.4 **a**
2008	3.4 ± 0.3 **b**	1.2 ± 0.2 **b**	0.27 ± 0.09	0.69 ± 0.17	1.1 ± 0.2 **b**
8-week period					
early Jun. to early Aug.	13.7 ± 2.0 **a**	7.9 ± 1.3 **a**	0.77 ± 0.12 **a**	1.43 ± 0.18 **a**	3.9 ± 0.4 **a**
early Aug to late Sep.	3.0 ± 0.3 **b**	1.3 ± 0.1 **b**	0.11 ± 0.01 **b**	0.39 ± 0.07 **b**	2.2 ± 0.4 **b**
late Sep. to late Nov	4.2 ± 0.6 **b**	0.3 ± 0.1 **b**	0.01 ± 0.01 **b**	0.15 ± 0.03 **b**	0.6 ± 0.2 **c**
Mass trap					
Control (no traps)	6.5 ± 1.2 **a** [1]	2.8 ± 0.9	0.36 ± 0.13	0.55 ± 0.15	2.0 ± 0.3
Vioryl	5.9 ± 1.5 **a** [1]	3.0 ± 1.0	0.17 ± 0.05	0.60 ± 0.13	2.3 ± 0.4
Pepito	8.6 ± 2.2 **a** [1]	3.2 ± 1.1	0.29 ± 0.11	0.59 ± 0.12	2.0 ± 0.4
Elkofon	6.8 ± 1.4 **a** [1]	3.5 ± 1.2	0.26 ± 0.08	0.87 ± 0.22	2.5 ± 0.5
ANOVA results (*p*-value)					

Table 6. *Cont.*

Factor	Bactrocera oleae (Olive Fly)	Lepidoptera (Butterflies and Moths)	Coleoptera (Beetles)	Hymenoptera (Parasitic Wasp Families [1])	Neuroptera (Net-Winged Insects)
Main effects					
Year (YR)	0.0153	0.0005	NS	NS	0.0025
8-week period (8WP)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Mass trap (MT)	0.0080	NS	NS	NS	NS
Interactions					
YR × 8WP	<0.0001	<0.0001	NS	NS	T
YR × MT	NS	NS	NS	NS	NS
8WP × MT	NS	NS	NS	NS	T
YR × 8WP × MT	0.0448	NS	NS	NS	NS

NS, not significant ($p > 0.1$); T, trend ($0.1 > p > 0.05$); means with the same lower case letter for the same factor within the same column are not significantly different according to Tukey's honest significant difference test (THSD; $p < 0.05$); [1], although ANOVA detected a significant effect of mass trap, no significant difference was detected when THSD tests were used to compare individual main effect means.

As expected, there were also a significant effect of year and sampling period on invertebrate activity profiles (Tables 5 and 6), given the differences in climatic background conditions between years and in different seasons during the year (Supplementary Figure S2).

A general trend was observed for the olive fly and all other invertebrate orders/families found in McPhail traps; activity increased between the first 8-week sampling period in winter and the second 8-week sampling period in spring (Table 5) and then decreased substantially over the summer (third and fourth sampling period) and remained low in the fifth sampling period in autumn (Table 6).

However, there was also a significant three-way interaction between year, 8-week sampling period and mass-trapping system after mass-traps were placed into experimental plots in June (Table 6). When this interaction was further analysed, significant differences in olive fly activity between mass-trapping systems were only detected in the early June to early August 8-week period in 2006, which was also the 8-week period that had the highest olive fly activity (Table 7); olive fly activity was significantly higher in plots with the Pepito traps compared with control, Vioryl, and Elkofon plots (Table 7). In addition, in 2006, olive fly activity decreased over time with the lowest olive fly activity recorded in last 8-week period (late September to late November (Table 7). In contrast, in 2007 and 2008, olive fly activity was lowest in the early August to late September 8-week period and increased again in the last 8-week period, although the increase was only significant in (a) the control plots and plots with the Pepito mass-trapping devices in 2007 (Table 7).

Table 7. Interaction means for the effects of year, 8-week monitoring period and mass-trapping system on olive fly activity determined by McPhail monitoring traps.

Factor 1	Factor 2	Factor 3. Mass-Trapping System			
Year	**8-Week Period**	**Control**	**Viroyl**	**Pepito**	**Elkofon**
2006	early June to early August	22.0 ± 5.0 **b A**	28.3 ± 5.4 **b A**	38.3 ± 11.4 **a A**	27.9 ± 3.8 **b A**
	early August to late September	4.0 ± 0.8 **a B**	3.5 ± 0.9 **a B**	4.8 ± 2.0 **a BC**	5.2 ± 0.9 **a BC**
	late September to late November	0.8 ± 0.1 **a C**	0.4 ± 0.1 **a B**	0.5 ± 0.2 **a C**	0.3 ± 0.2 **a C**
2007	early June to early August	9.6 ± 1.3 **a B**	5.8 ± 0.4 **a B**	9.2 ± 0.9 **a BC**	8.3 ± 1.0 **a B**
	early August to late September	2.5 ± 0.8 **a C**	1.3 ± 0.4 **a B**	2.2 ± 0.5 **a C**	1.8 ± 0.4 **a BC**
	late September to late November	8.5 ± 1.1 **a B**	6.9 ± 1.6 **a B**	10.6 ± 2.8 **a B**	7.2 ± 3.4 **a BC**
2008	early June to early August	3.9 ± 0.6 **a B**	3.1 ± 0.8 **a B**	4.6 ± 1.3 **a BC**	3.3 ± 0.6 **a BC**
	early August to late September	2.8 ± 0.4 **a BC**	1.8 ± 0.5 **a B**	3.2 ± 0.4 **a BC**	2.8 ± 0.8 **a BC**
	late September to late November	4.3 ± 0.5 **a B**	2.1 ± 0.7 **a B**	4.3 ± 0.8 **a BC**	4.3 ± 1.8 **a BC**

Means with the same lower case letter within rows and the same capital letters withing columns are not significantly different according to Tukey's honest significant difference test ($p < 0.05$).

3.3. Effect of Environmental Background Conditions on Invertebrate Activity

McPhail trap invertebrate activity data were also used for redundancy analysis (RDA) to study the effect of climatic explanatory variables/drivers (temperature, rainfall, humidity and wind direction) as drivers and the activity of invertebrate orders, suborders, families and the fruit fly pest species *Bactrocera oleae* (olive fly) and *Ceratitis capitata* (Mediterranean fruit fly) found in McPhail traps as response variables. In the biplot from the RDA, overall, 22.5% of variation is explained by axis 1 and a further 7.5% by axis 2 (the Eigenvalues were 0.225 and 0.075 for the x and y axes, respectively) (Figure 4).

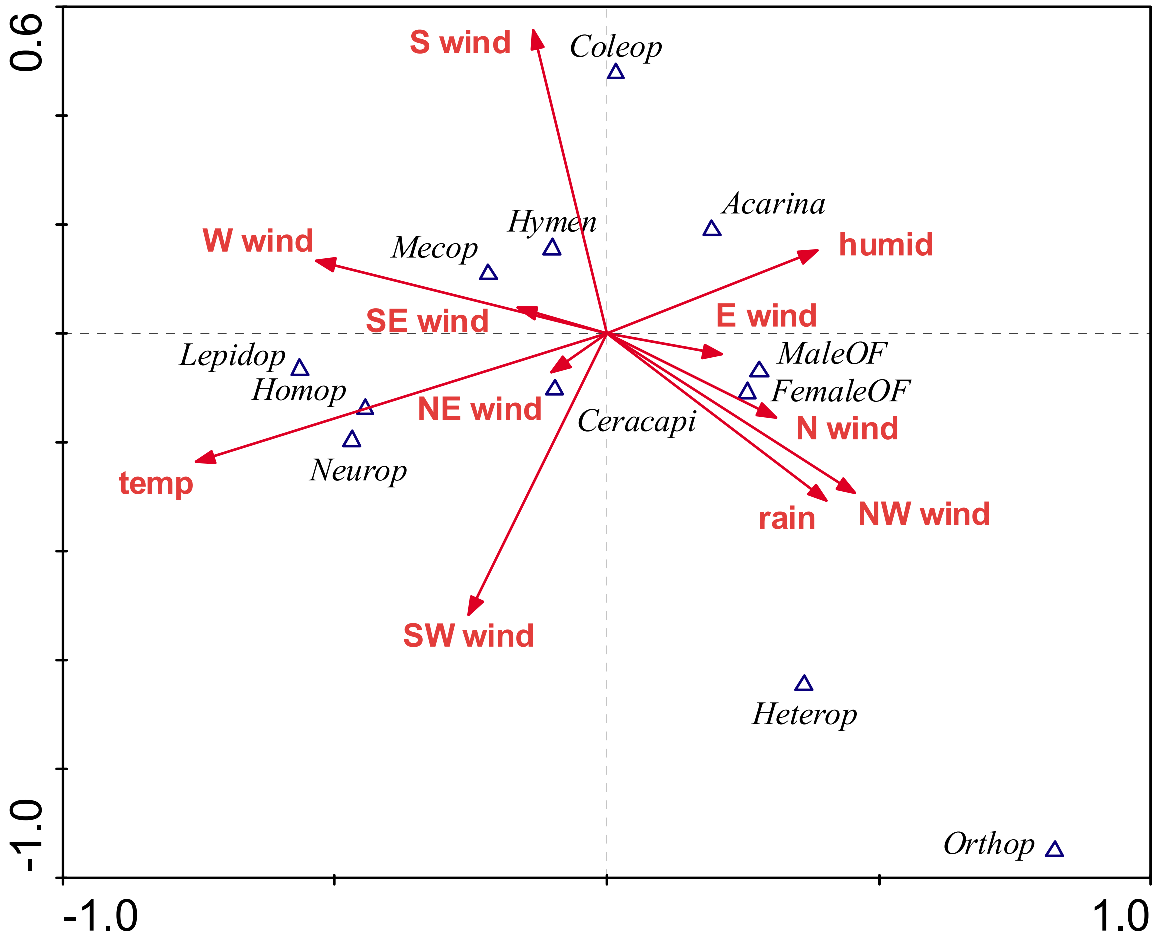

Figure 4. Bi-plot summarising results from the redundancy analysis (RDA) investigating associations between environmental parameters and the activity (number of animals recovered from McPhail traps) of different orders/species of invertebrates used to monitor olive fly activity in the experimental table olive orchards cv Kalamon used. Axis 1 explains 22.5% and axis 2 a further 7.6% of the variation. **Arrows** represent the environmental explanatory variables/drivers, and **triangles** represent the invertebrate orders and species response variables. **Explanatory variables/drivers:** Temp, temperature (F = 17.9; p = 0.002); rain, rainfall (F = 2.9; p = 0.026); humid, relative humidity (F = 2.7; p = 0.044); NW wind, northwest wind (F = 6.5, p = 0.002); E-wind, east wind (F = 6.0; p = 0.02), SW wind, southwest wind (F = 3.9, p = 0.04); S wind, south wind (F = 3.5; p = 0.028); W wind, west wind (F = 2.8; p = 0.044); SE wind, southeast wind (F = 2.3; p = 0.062), NE wind, northeast wind (F = 2.1; p = 0.060); N wind, north wind (F = 0.69; p = 0.592). **Response variables:** MaleOF, male olive flies; FemaleOF, female olive flies; Ceracapi, Mediterranean fruit flies (*Ceratitis capitata*); Acarina (mites and ticks); Coleop, Coleoptera (beetles); Heterop, Heteroptera (a group of families in the order Hemiptera, true bugs); Homop, Homoptera (suborder of the Hemiptera, true bugs); Hymen, Hymenoptera (parasitic wasp families of the order); Lepidop, Lepidoptera (butterflies and moths); Mecop, Mecoptera (Sorpionflies); Neurop, Neuroptera (net-winged insects); Orthoptera (grasshoppers, locusts, and crickets).

Temperature and wind direction were identified as the strongest drivers, but rainfall and humidity also explained a significant proportion of the additional variation (Figure 4). Olive fly, Acarina, Heteroptera and Orthoptera activity was positively associated with north and northwest and to a lesser extent east wind, and rainfall and humidity along the positive axis 1 and negatively associated with high temperatures and west wind and to a lesser extent south, southwest, southeast and northeast wind along the negative axis 1 (Figure 3).

In contrast, Coleoptera and to a lesser extent Hymenoptera activity was closely positively associated with south wind and Lepidoptera, Homoptera, Neuroptera and Mecoptera activity was positively associated with higher temperatures, west wind and to a lesser extent southeast, southwest and northeast wind (Figure 4).

4. Discussion

4.1. Effect of Different Cover Crops on Olive Fly and Non-Target Invertebrate Activity

The planting of ground cover crops in olive groves, fruit tree orchards and vineyards was previously reported to increase invertebrate biodiversity and the activity of pollinators and natural enemies of insect pests [36–39].

The establishment of legume or mixed legume/cereal cover crops in organic olive orchards during the winter rainy season is primarily carried out to increase nitrogen availability to olive trees and to suppress problematic weeds such as *Oxalis pes-caprae*, which is the main weed species found in olive orchards in the Messara region [7,19]. We have previously reported that (a) establishing cover crops using seeds of native *Medicago* species collected in olive orchards resulted in very low *Medicago* establishment (9 $\pm$ 1 plants/m^2), (b) that the Medicago treatment can therefore also be considered a "no cover crop control treatment, (c) that the Medicago/no cover crop control treatment resulted in the highest density of *Oxalis* (253 $\pm$ 11 plants/m^2), while cover crops established with a vetch/pea/barley mixture resulted in the lowest *Oxalis* plant density (134 $\pm$ 8 plants/m^2) and (d) that olive fly activity was very low in all cover crop plots, due to the use of commercial mass-trapping systems in the experimental olive orchard [7,19].

Results reported in this study show, for the first time, that cover crops had no detectable effect on all invertebrate orders and families monitored, except for three parasitic wasp families (Braconidae, Ichneumonidae, Pteromalidae) of the Hymenoptera. The finding that parasitic wasp activity was three times higher in vetch/pea/barley cover crop plots compared with the *Medicago*/no cover crop control plots suggests that the successful establishment of legume/cereal cover crop and/or the associated suppression of the weed Oxalis results in a substantial increase in the activity of three parasitic wasp families that contain a range of natural enemies of insect pests, including the parasitoid *Psyttalia concolor*, which is a natural enemy of the olive fly [16].

We did not quantify *P. concolor* activity in orchards, because invertebrates belonging to the Braconidae caught by suction sampling were not identified to species level in this study. In addition, since olive fly activity was very low, it was not possible to assess potential effects of higher parasitic wasp activity on olive fly activity.

4.2. Effect of Mass-Trapping Systems of Olive Fly and Non-Target Invertebrate Activity

Most commercially available mass-trapping products for olive fly control use a pheromone or food attractant to lure flies onto trap surfaces containing insecticides, and these products are widely used in organic production systems in Greece and other regions of the Mediterranean [19–22]. However, in this study, none of the trapping systems had a pheromone attractant.

Different to spraying chemosynthetic insecticides, the use of pesticide containing mass-trapping systems is thought not to result in a risk of generating pesticide residues in olive products and/or the environment and are therefore permitted for use in organic farming [18,19]. However, whether mass traps, similar to insecticide spray applications, have negative effects on the activity of non-target and beneficial invertebrate activity in olive orchards [14–16] has, to the best of our knowledge, not been studied previously. In addition, pheromone/insecticide-based mass-trapping systems are relatively expensive, and there has been concern that the use of pesticide-containing mass-trapping systems may affect demand for organic olive products because organic consumers expect organic foods to be produced without the use synthetic chemical pesticide products [28]. We have previously reported that two-food attractant-based mass-trapping systems (Pepito and Elkofon), which do not contain pesticides and kill olive flies by physically trapping them in a plastic bottle, can provide a similar level of fruit fly control when compared with an insecticide-based mass-trapping system (Eco-Trap) [19,40,41].

Results in this article report, for the first time, that the two bottle traps resulted in similar (a) low levels of olive fly fruit infestation, (b) numbers of olive flies caught within traps, and (c) olive fly activity within experimental plots (monitored with McPhail traps), but also (d) significant differences in the numbers of non-target invertebrates caught in traps. However, the majority of non-target pests caught in bottle traps were other Diptera (flies) and that no honeybees, bumblebees and parasitic wasp families of the Hymenoptera were found in the two bottle-based mass-trapping systems (Pepito and Elkofon), although parasitic wasp activity was detected by the McPhail traps used for monitoring of olive fly activity. This suggests that different to the use of pesticide sprays, which were shown to have negative effects of pollinators and the hymenopterous egg parasitoid *Trichogramma cacoeciae* (Marchal) [13,14], the use of the two plastic bottle-based mass-trapping systems had no negative impact on pollinators and natural enemies belonging to the parasitic wasp families of the Hymenoptera.

Our study did not allow for an assessment of the number of non-target invertebrates killed by the insecticide-based mass-trapping system (Eco-Trap), and it remains unclear whether the use pesticides in mass-trapping systems has a significant negative impact on beneficial invertebrates. However, univariate analyses of the McPhail monitoring trap invertebrate activity data identified no significant effect of mass-trapping system on any of the non-target invertebrate orders/families.

4.3. Effect of Environmental Background Conditions on Invertebrate Activity

The Messara region in Crete is known to have relatively low olive fly activity/pressure due to the high summer temperatures (>31 °C) which are known to result in high mortality of both larvae and adults [40–42]. The findings that olive fly activity was very low during the summer period in all three growing seasons and the negative association between temperature and olive fly activity identified by RDA were therefore as expected. The collapse of olive fly populations in summer and relatively low levels of activity during olive fruit maturation in autumn in the Messara region may therefore also explain why the pests can be efficiently controlled with mass-trapping systems and the high quality of olive oil produced there [18,19,41,42]. The finding of positive associations between olive fly activity and both rainfall and humidity found is also consistent with the results of previous studies [42–45].

Migration or movement of olive flies with the wind may contribute to the levels of olive fly in spring and summer in the olive orchard since abandoned olive orchards (in which no pest control treatments are used and fruits remain unharvested) and wild olives are known to be an important reservoir for adult olive flies, which may fly long distances and/or may be transported with the prevailing winds into commercial orchards in significant numbers [46–48]. This is likely to explain the positive association between north and northwest wind and olive fly activity identified by RDA in this study, since most abandoned olive orchards are located to the north and northwest of the experimental olive orchard in the foothills of the Psiloritis mountain range. In contrast, intensively managed, commercial olive orchards (in which olive flies are controlled by regular insecticide sprays) are located southeast, south, southwest, and west of the experimental field, which is likely to explain the negative associations between these wind directions and olive fly activity identified by RDA.

Uniquely, our study also assessed associations between environmental parameters and the activity of invertebrates other than olive fruit flies found in McPhail traps. Results suggest that except for mites and ticks (Acarina) and true bugs belonging to the Heteroptera (Hemiptera), the environmental activity profiles were different to those observed for olive fruit flies. Most importantly, there were positive associations between higher temperatures, and butterflies and moths (Lepidoptera), Homoptera (Hemiptera), net-winged insects (Neuroptera) and to a lesser extent scorpion flies (Mecoptera) and parasitic wasps (Hymenoptera).

In addition, Lepidoptera activity was strongly positively associated with west and to a lesser extent south, southwest and southeast wind; this may have been due to large areas of field and greenhouse vegetable production being located to the west, south, southwest, and southeast of the experimental olive orchard. Many Lepidoptera species are common pests in vegetables [49], and Lepidoptera activity is known to be high around vegetable fields/greehouses in Messara (Kolaros, D. Hellenic Mediterranean University, Heraklion, Greece; personal communication).

Activity of invertebrates belonging to three parasitic wasp families of the Hymenoptera, which include *Psyttalia concolor* Szlepligeti (synonym *Opius concolor*, Hymenoptera, Braconidae), the major parasite of *Bactrocera oleae*, was found to be positively associated with southerly wind. This may indicate that the intensive organic and conventional olive production in the Messara plain and/or the Asterousia mountains, which is located to the south of the experimental orchard in the Messara valley, provides a reservoir for potentially beneficial Hymenoptera species. However, the parasitic wasp found in McPhail monitoring traps was not identified to the species level. The effect of environmental parameters that affect the population dynamics of parasitic wasps in olive orchard-dominated agricultural ecosystems should therefore be investigated in more detail in future studies, since such information may be used to further improve decision support systems (DSS) for the integrated control of olive fruit flies [47,48].

4.4. Study Limitations

The main limitation of this study was the low olive fly activity and associated low fruit infestation in the two olive fruiting seasons monitored/assessed in this study. It was therefore not possible to perform an accurate comparison of the efficacy of the three mass-trapping systems for olive fly control. The experiments should therefore be repeated in high olive fly pressure environments (e.g., northern regions of the Mediterranean with lower summer temperatures).

Until confirmed in future studies, trends ($0.1 > p < 0.05$) and significant effects with relatively high p values ($p > 0.01$) should be interpreted with caution, due to the risk of type 1 errors given the relatively high number of invertebrate activity parameters assessed.

Although vacuum sampling is an established method to compare invertebrate activity in contrasting environments, it is possible that the efficiency in collecting invertebrates with the vacuum sampler may have been affected by the botanical composition and canopy structure of different cover crops and that this had a confounding effect of the estimates of invertebrate activity.

Another limitation was that impacts of mass-trapping on non-target invertebrates could only be assessed for the two bottle traps, because they retained the invertebrates that were lured into the trap. Furthermore, it was not possible to identify potential benefits of the higher parasitic wasp populations resulting from cover crops established from a vetch/pea/barley seed mixture (or associated lower *Oxalis* weed density on olive fly activity and fruit infestation levels). The efficacy of the three different mass-trapping systems and their impact on non-target and beneficial insect populations should therefore be investigated in regions of the Mediterranean with higher olive fly pressure in the future.

This recommendation is supported by the findings of (a) significantly lower olive fly activity in Eco-Trap protected plots compared with plots protected by Pepito traps in the early June to early August 8-week period in 2006 and (b) the trend towards lower fruit infestation in plots protected by Elkofon compared with plots protected by Pepito traps in August 2006, because it indicates that there may be differences in trap efficacy at higher olive fly pressure. However, in areas with larger organic olive fields, a different Eco-Trap mass-trapping system is used where in addition to the food attractant, an olive fly-specific pheromone is also incorporated into traps [20–22,50]. This may significantly increase the efficacy of the Vioryl mass-trapping system in areas where the use of these traps is feasible. However, due to (a) higher cost of the pheromone attractant containing traps, (b) the small size of most organic olive orchards and because (c) most organic orchards are surrounded by conventional fields in the Messara plain, the current advice to organic farmers in the Messara region is to use Vioryl traps which contain the food attractant only or one of the two-plastic-bottle traps compared in this study (Kolaros, D. Hellenic Mediterranean University, Heraklion, Greece; personal communication).

It was not possible to determine the reasons for the poor *Medicago* germination rate and establishment. This may have been due to (a) seed dormancy, which is common in *Medicago* species [51,52], not having been broken in a large proportion of seed or (b) seeds having been infected with fungal pathogens while lying on the soil surface before being collected [53].

5. Conclusions

This study demonstrated that, compared to the control (poorly established *Medicago*), the successful establishment of mixed legume/cereal cover crops can increase the activity of parasitic wasps belonging to the families Braconidae, Ichneumonidae and Pteromalidae, which include a range of natural enemy species of invertebrate pests. If future studies show that the increase in parasitic wasp activity contributes to the control of invertebrate pests in olive orchards (e.g., olive flies) or neighbouring vegetable fields, this would add a new "ecosystem service" to the list of known benefits from cover crops in olive orchards (e.g., weed suppression, improved nitrogen availability, reduced soil erosion).

Our study also provided evidence that the use of two-plastic-bottle mass-trapping systems for olive fly control has no substantial impact on non-target and in particular potentially beneficial insects such as pollinators and parasitic wasps.

Consumer perceptions that organic farming methods deliver biodiversity benefits are an important driver of demand for organic products [27,28]. Since results suggest that (i) the use of cover crops (which is common practice in organic but not conventional farming) results in an increase in parasitic wasp activity and (ii) mass-trapping systems (which are widely used by organic but not conventional farmers) have no negative effect on beneficial insects (parasitic wasps, bees, bumble bees), the study provides further evidence for environmental benefits from organic farming. This may reinforce existing consumer perceptions and further stimulate demand for organic foods. In addition, the information provided may encourage some conventional olive producers to use ground cover crops and/or mass-trapping systems as part of their integrated crop management plan.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12102576/s1, **Supplementary Figure S1:** Experimental cover crop plot dimensions. **Supplementary Figure S2:** Average mean daily temperature and total rainfall per months in the three cover crop growing seasons monitored.

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