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Evaluation of the Impact of Plant Protection Products (PPPs) on Non-Target Soil Organisms in the Olive Orchard: Drone (Aerial) Spraying vs. Tractor (Ground) Spraying

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Abstract: Policies aimed at reducing plant protection products (PPPs) are part of the UN's 2030 Agenda for Sustainable Development. Sustainable management of PPPs is crucial for soil health, biodiversity, and ecosystem services, including food provision. While PPPs can control pests and enhance agricultural yields, they also pose environmental and health risks by contaminating water, soil, and non-target organisms through airborne drift. Investigating innovative and more sustainable distribution methods can support sustainability goals. This study aimed to evaluate the potential impact of the pesticide Spintor® Fly on non-target soil organisms in olive orchards comparing two spraying methods: a traditional Casotti® pump mounted on a tractor and an innovative Unmanned Aerial Vehicle (UAV) developed for the project. The study was conducted in 2021 in an organic olive orchard, which was divided into two plots: a Casotti-treated plot (CAS) and a drone-treated plot (DRO). A strip of uncultivated land at the edge of the orchard was used as a (non-treated) control plot (CAP). The impact on native soil microarthropod communities was assessed using the arthropod-based Soil Biological Quality Index (QBS-ar) and Bait Lamina Test (BLT). Soil samples were collected for earthworm avoidance tests and soil chemical–physical analysis. The results obtained with QBS-ar and BLT indicated no significant differences between DRO and CAS, in both sampling periods (pre- and post-treatment). However, DRO generally exhibited slightly better performance than CAS. The avoidance behaviour was confirmed for both CAS and DRO, although it was lower for the latter. Overall, drone aerial spray performed slightly better, suggesting a potentially lower impact on soil communities. Our results provide initial clues for the sustainable use of drones in agriculture with no increased risks for soil health compared to traditional methods. Further long-term studies should be conducted to validate these findings and possibly confirm the long-term benefits of drone applications compared to traditional methods.

Keywords: Spintor® Fly; drone; soil biodiversity; QBS-ar; bait lamina test; avoidance test

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1. Introduction

Italy is one of Europe's leading olive oil producers, with annual production reaching 328,000 tonnes in the 2023/24 crop year [1]. However, the production volume has fluctuated considerably, with a recent decline attributed to exceptionally hot weather and prolonged drought conditions [1–3]. Studies suggest that climate change will exacerbate climate stress on olive production and alter trophic interactions of olive and its obligate pest, the olive fruit fly (*Bactrocera oleae* Rossi, 1790) [2]. To control the olive fruit fly, there are many agrochemicals available on the market, some based on the active ingredient spinosad combined with a protein-attracting food bait, such as the commercial formulation Spintor® Fly (Corteva, Agriscience, Milano, Italy), which is approved for use in organic farming.

The use of spinosad-based PPPs has grown rapidly over the past decade and it is one of the most widely used natural bioinsecticides in the world [4]. Based on numerous toxicological and in field studies, spinosad has been classified as an environmentally and toxicologically reduced-risk compound [5,6]. According to the Bio-Pesticide DataBase (BPDB), spinosad has a low toxicity to mammals and birds [7]. It is moderately toxic to earthworms but highly toxic to honeybees. However, the US National Pesticide Information Center (NPIC) reports that spinosad has little if any effect on honeybees and other beneficial insects after drying [6]. Spinosad is not considered a highly persistent, bioaccumulative, and toxic (PBT) substance (vPvB) [7]. The dissipation time (DT50) of spinosad in soil generally ranges from 2.0 to 7.8 days, depending on climate, season, soil type, pH, crop type, dose and application method [8–11]. Spinosad-based pesticides have a favourable environmental and ecotoxicological profile. Optimising their targeted application and minimising drift to non-target areas will enhance environmental safety, reduce usage costs, and promote economic, social, and environmental sustainability. Unmanned Aerial Vehicles (UAVs) provide an efficient and cost-effective solution for aerial spraying of PPPs. They can fly at low altitudes, hover near plant canopies, and navigate challenging terrains using pre-programmed flight paths. Studies show UAV spraying is effective for various crops, including olive orchards in the mediterranean basin [12–15]. This method reduces environmental impact by optimising the quantity and distribution of PPPs on the tree canopy, thus avoiding drift to other compartments. Drift is especially significant when it comes to pesticides, as their effects extend beyond controlling pathogens—which they were originally designed for—to include potential risks to human health and non-target organisms, particularly soil biodiversity [16]. This largely neglected component of biodiversity plays a key role in supporting multiple ecosystem functions and services, including nutrient cycling, climate regulation, pest control, and food production [17], but it is less considered than above-ground biodiversity in studies analysing the impacts of PPPs in agroecosystems. Beaumelle et al. (2023) [18] recently highlighted that the unsustainable and inappropriate use of PPPs can adversely affect soil communities and the ecosystem services they provide. In this respect, innovative aerial spraying technology (using a UAV) can help to reduce the impact of PPP on soil biodiversity. However, to date, the distribution of PPPs in orchards is generally achieved using backpack pumps or tractor-mounted pumps, which have much lower application efficiency. In fact, according to the Directive 2009/128/EC, the application of PPPs by UAV is generally prohibited in Europe, although Member States may ask for derogations [19]. One of the critical limitations for the application of new aerial spraying technologies is the stringent regulatory scheme. European legislators do not want to underestimate the risks to human and environmental health posed by using drones [20]. In this context, the Smart Farming: Innovating the Environment with Drones (S.F.I.D.A.) Project (Marche Region, RDP 2014/22—M 16.1.A.2, ID 29073) [21] has the objective to develop an aerial spraying system using a drone to distribute PPPs authorised in organic farming for the control of olive tree adversities in specialised plantations. The aim was to evaluate the environmental impact of the distribution of PPPs through the implementation of Precision Agriculture (PFA) techniques in conjunction with an examination of the regulatory issues pertaining to the aerial application of PPPs by drones, with consideration of the EU legal framework.

Under the S.F.I.D.A. project [21], this study aims to evaluate and compare the effect of innovative aerial spraying technology (using a UAV—for the sake of simplicity in the rest of this article, the term 'drone' will be used) and conventional ground spraying technology (using a Casotti® pump mounted on a tractor) for the application of Spintor® Fly in olive orchards. We hypothesised that the use of a drone would be less impactful compared to the traditional method on soil biodiversity. In this regard, to the best of our knowledge, no studies have yet evaluated the impact of the bioinsecticide Spintor® Fly on non-target soil organisms in a field experiment. Furthermore, no studies have assessed and compared the effects of two different Spintor® Fly distribution systems on soil fauna in olive orchards. The arthropod-based Soil Biological Quality Index (QBS-ar) and ecotoxicological tests, including the earthworm avoidance test (ISO Standard 17512-1:2008) [22] and the Bait Lamina Test (BLT) (ISO Standard 18311:2016) [23], were applied at this scope.

2. Materials and Methods

2.1. Set-Up of Drones and Ground Distribution Systems and Spintor® Fly Spraying and Monitoring Schedules

The drone was manufactured specifically for the SFIDA project to spray Spintor® Fly on the canopy of olive trees. Briefly, it is a BLY-C aircraft (DJI Matrice 600 PRO) in AGRI version with a Liquid Distribution Kit and 10-litre tank (Aermatica3D, Como, Italy) [21]. The dispensing system was equipped with a 0.2 mm Casotti® nozzle (F. Allegrini, A. Feliziani, M. Coletta, A. Passacantando, A. Fioretti, A. La Terza, manuscript in preparation). The conventional distribution system (from the ground) was a Casotti® pump FAMILY 30 (Casotti, Parma, Italy) mounted on a tractor (Kubota M8540 Power Krawler, Kubota Italia, Milano, Italy).

Spintor® Fly is an insecticide bait based on spinosad and specific attractants for the control of *Diptera tephritidae*. The main ingredient, spinosad, consists mainly of spinosyns A and D and is a fermentation product of the actinomycete *Saccharopolyspora spinosa* [24]. Spintor® Fly was used according to the manufacturer's instructions (Corteva, Agriscience, Milano, Italy); a solution consisting of 1 L Spintor® Fly diluted in 4 L water (for a final Spinosad concentration of 0.06 g/L) was prepared for the treatment of 1 ha (5 L mixture/ha).

In both cases, the application was conducted at low pressure (2–3 atm) without product nebulization. Large drops (4–6 mm) were generated from the nozzles. A total of eight applications were carried out from 6 August to 24 September 2021, with an interval of 7 days between each administration. The application took place on the same days for both distribution methods (Supplementary Material—Video S1).

2.2. Study Site and Climatic Data

The study was conducted in a high-density experimental olive orchard, under organic management since it was planted, located at the Agricultural School G. Garibaldi (province of Macerata, central Italy; N 43° 17′ 18.9096, E 13° 25′ 10.3548). Different olive tree varieties were present in the orchard, including Piantone di Mogliano, Leccio del Corno, Arbequina, Sikitita, and Koroneiki. The olive orchard was divided into two main experimental plots of 0.25 ha each: a Casotti pump-treated plot (CAS) and a drone-treated plot (DRO). A strip of uncultivated land of 0,11 ha at the edge of the orchard (on the north-eastern border of CAS) was used as a (non-treated) control plot (CAP) (Figure 1).

The area of Macerata is situated within a temperate climatic zone [25], as it is characterised by a mean temperature exceeding 10 °C during the hottest month and a temperature ranging between 0 and 18 °C in the coldest month [26]. To describe the climatic condition of the experimental site located at the Agricultural School G. Garibaldi, a thermo-pluviometric diagram was generated using average temperature and total rainfall data for the year 2021 provided by the "Agenzia per l'Innovazione nel Settore Agroalimentare e della Pesca delle Marche" (AMAP) (Jesi, Italy) [27] (Figure 2). Data were recorded by the Treia weather station (Macerata, Italy), which is near the study site and with a similar altitude.

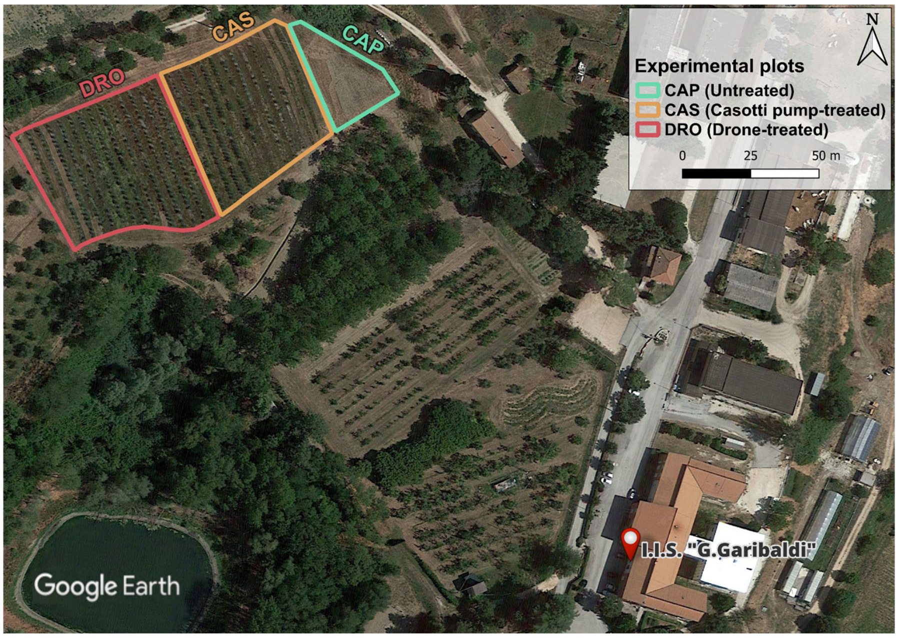

Figure 1. Subdivision of the experimental plots. DRO = drone-treated plot; CAS = Casotti pump-treated plot; CAP = untreated plot. Map made with QGIS software (v. 3.34.12-Prizren) and using 2021 satellite image from Google Earth Pro software, v. 7.3.6.

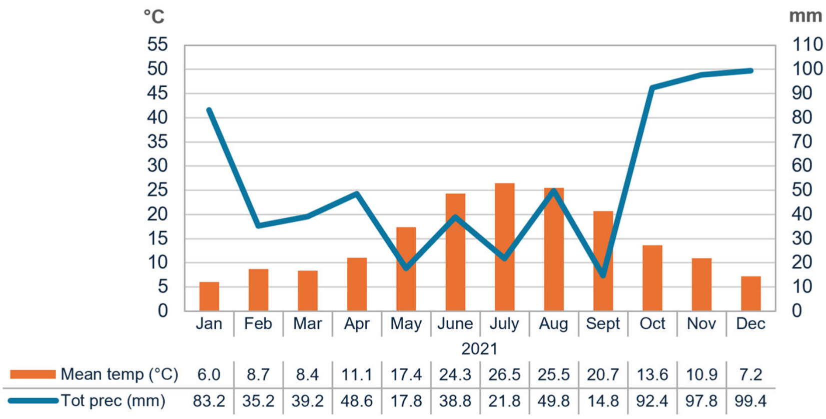

Figure 2. Thermo-pluviometric diagram of the study area in 2021.

2.3. Soil Chemical Analysis

Soil samples for chemical–physical analyses were analysed by the laboratory of the "Agenzia per l'Innovazione nel Settore Agroalimentare e della Pesca "Marche Agricoltura Pesca" (AMAP)", Jesi (Italy) [27] and collected in accordance with the official methods for soil analysis of the Italian legislation DM 13 September 1999 SO No. 185 (Approvazione dei "Metodi Ufficiali di analisi chimica del suolo") [28]. In each plot (CAP, CAS and DRO), 10 soil sub-samples were collected by using a pedological auger and then mixed into a single composite sample (1 Kg). Sub-samples were collected randomly within the CAP area, and in the olive grove, the soil was collected from the inter-row. The soil analysis was carried out before and after the Spintor® Fly application. The pre-treatment sampling was conducted on 30 July, while the post-treatment sampling was conducted on 25 September. The analysed parameters were total limestone, TL (g/Kg); cation exchange capacity, CEC (meq/100 g); electrical conductivity, EC (dS/m); total organic carbon, TOC (g/Kg); total nitrogen, TN (g/Kg); assimilable phosphorus, P (mg/Kg); assimilable iron, Fe (mg/Kg); assimilable copper, Cu (mg/Kg); assimilable zinc, Zn (mg/Kg); and assimilable manganese, Mn (mg/Kg). In addition, soil analyses for the detection of spinosad, spinosyn A and spinosyn D were carried out by Bucciarelli Laboratori S.r.l. (Ascoli Piceno, Italy) using the AOAC method 2007.01 2013 [29]. The substances were measured through acetonitrile extraction and analysis by gas and liquid chromatography (GC and LC) coupled with mass spectrometry (MS) and tandem mass spectrometry (MS/MS). The limit of detection for the method was 0.05 mg/Kg.

2.4. The Arthropod-Based Biological Soil Quality Index (QBS-ar)

The arthropod-based Biological Soil Quality Index (QBS-ar) [30,31] was used to assess the overall soil health condition of the different plots. The index is based on the identification of the main groups of microarthropods (e.g., isopods, mites, collembola, etc.) and on the assignment of an EMI value (Ecomorphological Index), which ranges from 1 to 20, depending on their degree of adaptation to soil life. The sum of the EMI represents the soil quality indicator itself (QBS-ar value). Three QBS-ar samples, each comprising three subsamples of soil cores equally spaced (10 m) along the slope, were collected at each plot. In CAS and DRO plots, the samples were collected in the central part of different inter-rows of the olive grove, away from the edge. In the CAP plot, the samples were collected in the central part. The subsamples were collected by using a steel core drill to obtain an equivalent volume of soil (approximately 800 cm^3). The samples were taken to the laboratory and placed (within 24 h) in a Berlese-Tullgren selector for the extraction of soil microarthropods (extraction time: 7 days). The QBS-ar value was obtained by summing the highest EMI values attributed to each biological form (BF) in the three subsamples (A, B, C). Soil samples were collected on 30 July (pre-treatment) and on 25 September (post-treatment).

2.5. Bait Lamina Test

The Bait Lamina test (BLT) (ISO standard 18311:2016) [23,32,33] is used to evaluate the feeding activity of soil micro-, meso- and macro-organisms in situ. Small hole-bearing plastic strips (160 mm $\times$ 5 mm $\times$ 1 mm), with bait-filled holes of 1.5 mm Ø, separated 5 mm from each other, were used for the test directly in the selected olive orchard. The holes contained a bait consisting of a mixed powder of 70% cellulose, 27% bran flakes, and 3% active charcoal (Terra Protecta Ökosystembewertung GmbH, Berlin, Germany), which was mixed with tap water to obtain the proper texture. The strip holes were manually filled with bait two times to avoid gaps. The strips were inserted vertically into the soil and left in place for two weeks. Afterwards, the strips were collected, taken to the laboratory, and placed on a light table. A visual assessment was made, photographs were taken, and the percentage of mixture consumed was calculated. The percentage of the bait area consumed in each hole ranged from 0 (i.e., hole full of bait) to 100 (i.e., empty hole) within a five-point scale (0, 25, 50, 75, and 100) [31]. Three sets of 10 bait-filled strips (each strip bears sixteen holes) were placed in each of the three plots (CAS, DRO, and CAP). In the CAS and DRO plots, each set of strips was placed in the central part of different inter-rows of the olive orchard, away from the edge. In the CAP plot, the three sets of strips were placed at equal horizontal distances along the central part. In the pre-treatment sampling, the strips were placed on 19 July and removed on 2 August, while in the post-treatment, the strips were placed on 20 September and removed on 4 October.

2.6. Avoidance Test

The avoidance test (ISO standard 17512-1:2008) [22] was conducted using adult earthworms of the *Eisenia fetida* and *Eisenia andrei* species [34]. It is a soil ecotoxicology test that is widely employed for the assessment of the effects of diverse types of contaminants (including pesticides) on earthworm behaviour. The test employed a two-section plastic tray, separated by a thin plastic divider. The soil collected from the un-treated site (CAP) was used as control soil, while the soils collected from the treated plots (CAS and DRO) were used as test soils. For each plot, 10 soil samples were collected with a stratified random sampling approach (two samples randomly taken every two rows, one in the upper part of the slope and one in the lower part, with the exclusion of areas less than 5 m from the borders), which were then pooled together. All soils were sieved at 2 mm before use. Two days before the test, adult earthworms (300–600 mg body weight) were selected and placed in a box with the sieved control soil (CAP), moistened at 60% of its water holding capacity using demineralised water, for acclimatisation. At the beginning of the test, one-half of the trays was filled with control soil (CAP), while the other half was filled with test soil (CAS or DRO). In the test, six replicates were used for controls, namely trays filled in both halves with control soil (CAP), five trays in which the control soil was tested against the CAS plot soil, and five trays in which the control soil was tested against the DRO plot soil. The soils were moistened to 60% water holding capacity using demineralised water. Once the trays had been prepared, the divider was removed, and 10 adult earthworms were placed in the centre of the furrow. The trays were placed in a growth chamber (GC401 NUVE, Steroglass, San Martino In Campo, Italy) and maintained under controlled conditions for 48 h: 50% moisture and a temperature of 20 ± 2 °C, photoperiod of 16 h of light and 8 h of darkness. The avoidance value was calculated as follows:

$$x = \left(\frac{nC - nT}{N}\right) \times 100$$

where x is avoidance, expressed as a percentage; nC is the number of worms in the control soil (either per vessel or in the control soil of all replicates); nT is the number of worms in the test soil (either per vessel or in the test soil of all replicates); and N is the total number of worms (10; either per vessel or in the control soil of all replicates), as reported in the ISO 17512-1:2008 [22]. The test is considered valid if the number of dead or missing worms is <10% per treatment and if the distribution in the control is within a 40:60% ratio. The avoidance test was applied with soil samples collected post-treatment only, on 25 September.

2.7. Statistical Analysis

The QBS-ar data were first checked for normality of distribution (Shapiro–Wilk test), differences between plots (CAP, CAS and DRO) were evaluated through One-way ANOVA and Tukey's honestly significant difference post hoc test, whereas differences between the same plot in pre- and post-treatments were evaluated through Student's t test or Wilcoxon test, according to distribution. The Bait Lamina Test (BLT) and the avoidance test data were evaluated through One-way ANOVA and significant differences in pairwise comparisons were tested through Student's t test with Bonferroni correction. A p-value < 0.05 has been considered significant. A one-tailed Fisher's exact test was used to detect possible deviations in avoidance behaviour in treated soils, while a two-tailed test was used to check the validity of the control test. All statistical analysis was performed using R software, version 4.3.2 [35].

3. Results

3.1. Soil Chemical and Climatic Data Analysis

In Table 1, the results of the soil chemical analysis are reported. In all cases, the soil was characterised by a high concentration of Ca. The cation exchange capacity and electrical conductivity were very similar in the various plots, in both pre- and post-treatments. The total organic carbon and total nitrogen contents were higher in CAS and DRO than in the CAP in both cases. The content of assimilable Fe, Cu and Zn appeared low in all plots and in both pre- and post-treatments. In contrast, the assimilable P content appears high in CAS and CAP and medium in DRO, while assimilable Mn content appears high in DRO and low in CAP and CAS, both pre- and post-treatment. The chemical and physical composition of the soil was very similar both in the three plots and in the pre- and post-treatment period. Spinosad, Spinosin A, and Spinosin D were not detectable in all the investigated soil samples.

The thermo-pluviometric diagram (Figure 2) revealed that the histograms of the average temperatures indicated a dry period from May to September. Rainfall increased significantly from the end of September to October and remained intense and constant during the following months, which were characterised by a surplus of water. The pre-treatment sampling activities (July–August) took place during a period of drought, while the post-treatment sampling activities were carried out when weather conditions had improved (September–October).

Table 1. Results of soil chemical–physical analysis pre- and post-treatment. Total limestone, TL; cation exchange capacity, CEC; electrical conductivity, EC; total organic carbon, TOC; total nitrogen, TN; assimilable phosphorus, P; assimilable iron, P; assimilable copper, Cu; assimilable zinc, Zn; assimilable manganese, Mn.

Treatment	Plot	TL (g/Kg)	CEC (meq/100 g)	EC (dS/m)	TOC (g/kg)	TN (g/kg)	P (mg/Kg)	Fe (mg/Kg)	Cu (mg/Kg)	Zn (mg/Kg)	Mn (mg/Kg)
Pre-treatment	CAP	310	19.9	0.885	13.63	1.55	17.9	9.6	1.59	0.93	9.6
	CAS	289	17.9	0.873	20	2.78	17.8	7.23	1.63	1.21	12.63
	DRO	253	21.3	0.922	19.5	2.75	15	9.2	1.87	1.20	15
Post-treatment	CAP	305	20.3	0.899	12	1.65	19.8	10	1.75	0.73	8.5
	CAS	285	18.4	0.871	19.7	2.65	18.6	6.65	1.7	1.29	8.4
	DRO	257	21	0.924	22.15	2.7	13.4	9.4	1.9	1.15	14.4

3.2. QBS-ar Evaluation

The analysis of the QBS-ar values registered in each plot showed no statistically significant differences between and within all the analysed plots, pre- and post-treatment (Table 2, Figure 3).

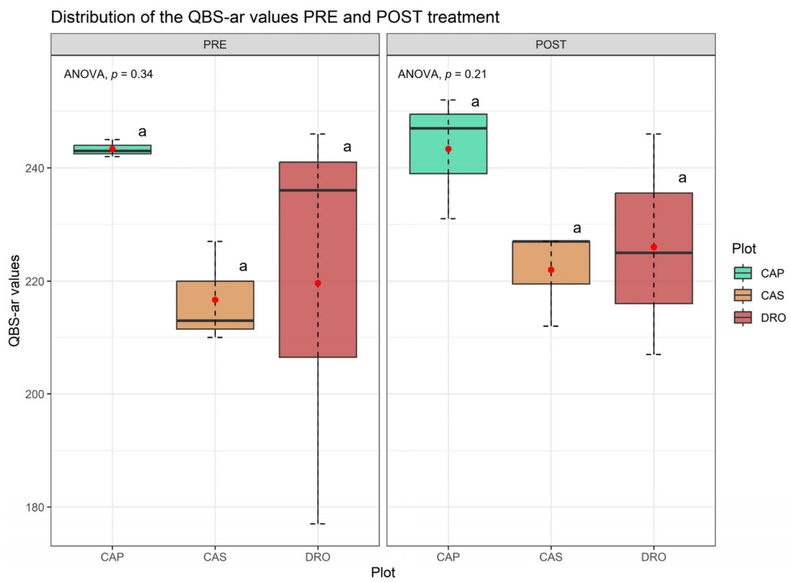

Figure 3. Distribution of the QBS-ar values in the three plots. The median (horizontal line), mean (red dot), and error bars (dashed line) are reported. Lowercase letters represent significant differences by Tukey's honestly significant difference post hoc test (comparison among plots within the same period) at *p*-value < 0.05. The comparison within the same plot in pre- and post-treatments was made by Student's *t* test and no significant differences were found.

In both samplings (pre- and post-treatment), the QBS-ar values in the untreated plot (CAP) were consistently, but not significantly, higher than those obtained in the treated plots (CAS and DRO). In both treated plots, there was a slight increase in values in the post-treatment. In both samplings (pre- and post-treatment), the plot treated with the drone (DRO) always showed slightly higher values than the plot treated with the Casotti® pump (CAS).

Table 2. Average QBS-ar values in each plot. Lowercase letters represent significant differences among plots pre- and post-treatment by Wilcoxon (CAS) or Student's *t* tests (CAP and DRO) at *p*-value < 0.05.

	QBS-ar Values	
Plot	**Pre-Treatment**	**Post-Treatment**
CAP	243 a	243 a
CAS	217 a	222 a
DRO	220 a	226 a

3.3. Bait Lamina Test

The BLT highlighted better performance of the CAP plot compared to the treated plots, and the absence of differences between the latter (CAP vs. DRO) (Figure 4, Table 3). In detail, in the pre-treatment sampling, CAP exhibited an average consumption of 28.91% (σ = 23.85) of the mixture, in comparison to 11.25% (σ = 12.98) in the drone-treated plot (DRO) and 11.91% (σ = 11.26) in the tractor-treated plot (CAS). The results of the pairwise t-test showed statistically significant differences between the control plot (CAP) and treated plots (CAS and DRO) and no statistically significant differences between CAS and DRO (Figure 4a). In the post-treatment sampling, an average consumption of 38.44% (σ = 26.62) of the mixture was registered in CAP, while a 21.93% consumption (σ = 25.34) was reported for DRO and a 20.83% (σ = 16.77) for CAS. Similarly, significantly higher values were observed in the control plot (CAP) compared to treated plots, while no significant differences were observed between treated plots (CAS and DRO). However, while not significant, the feeding rate was higher in DRO than in CAS in the post-treatment sampling (Figure 4b). Indeed, the comparison between pre- and post-treatment values for each plot resulted in the detection of a significant increase in the feeding activity in DRO only (Figure 4c).

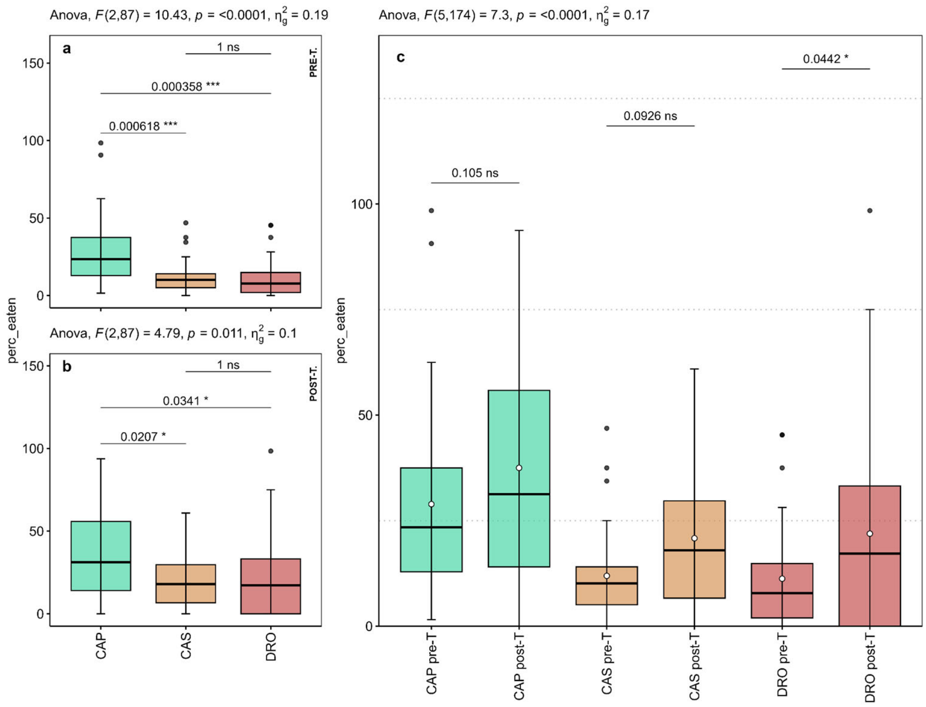

Figure 4. Boxplots of percent values of eaten bait. Comparison of eaten bait percentages between treatments among pre- (**a**) and post-treatment (**b**) samples, and between pre- and post-treatment samples of the same application mode (**c**). On top of the plots, the results from the ANOVA tests are shown: "ns" indicates not significant differences ($p > 0.05$); "*" $p \leq 0.05$; "***" $p \leq 0.001$; isolated black dots represent outliers. Adjusted *p*-values shown in the charts refer to the pairwise Student's *t* test results after Bonferroni correction.

Table 3. Results of the Bait Lamina Test (BLT). The mean percentage of bait consumed is presented. The mean bait consumption indicates the number of holes consumed, calculated based on the total number of holes present in the strip (16).

Sampling	Plot	Mean Bait Consumption (%)
Pre-treatment	CAP	28.9
	CAS	11.9
	DRO	11.3
Post-treatment	CAP	37.5
	CAS	20.8
	DRO	21.9

3.4. Avoidance Test

The validity criteria were met for this test (mortality rate < 10% and no statistically significant avoidance behaviour in the controls). As illustrated in Figure 4, when the control soil (CAP) was used on both sides of the tray, no preference was detected between the two sides (Fisher's exact test, p-value = 0.1033). In contrast, when the control soil (CAP) was compared to that of the treated plots (CAS and DRO), a marked tendency to avoid the latter soils was observed. The avoidance behaviour was confirmed for both CAS and DRO by Fisher's exact test, which resulted in a p-value of 0.0008 and 0.0020, respectively. The analysis of the results obtained in the CAS and DRO application modes showed a statistically similar result, although with a lower mean avoidance value for the drone-treated plot: 32% for DRO (σ = 22.8) and 36% (σ = 26.07) for CAS (Figure 5, Table 4).

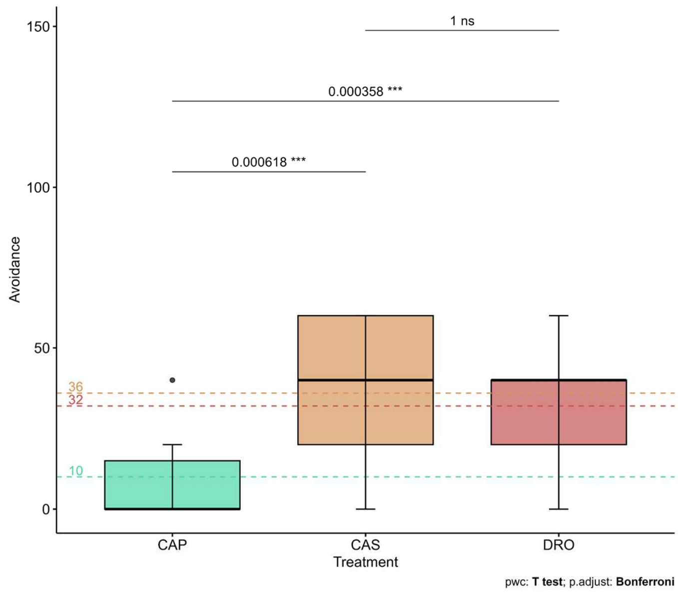

Figure 5. Boxplot of the distribution of avoidance values. Higher values indicate a greater tendency to escape towards the control soil (CAP). Dashed lines and values on the left side of the plot represent the mean avoidance values, p-values shown at the top represent the significance level obtained from Student's t-test. "ns" indicates not significant differences ($p > 0.05$); "***" $p \leq 0.001$; isolated black dots represent outliers.

Table 4. Results of avoidance test. Avoidance values are represented as percentages. Lowercase letters represent significant differences among plots by Student's t test at p-value < 0.05.

Application Mode	CAP	CAP	CAP	CAP	CAP	CAP	CAS	CAS	CAS	CAS	CAS	DRO	DRO	DRO	DRO	DRO
Avoidance (%)	40	0	0	0	20	0	60	40	20	60	0	40	60	20	0	40
Mean avoidance per application mode (%)	10 a						36 a					32 a				

4. Discussion

The QBS-ar index, based on the evaluation of the soil microarthropods community, was higher in the control plot (CAP) compared to both treated plots (CAP and DRO). This outcome was expected, as the CAP plot, unlike the olive grove, is a semi-natural environment not affected by agricultural practises. However, the overall soil biological quality in the olive grove was found to be excellent in all plots and in both pre- and post-treatment samples, with values exceeding the threshold (93.7) set for high biological quality in agricultural soils [30,36], indicating an excellent soil quality for the area. No significant differences were observed between CAP and the treated plots (CAS and DRO). However, the QBS-ar values of the drone-treated plot (DRO) were slightly higher than those of the plot treated with the tractor-mounted Casotti pump (CAS) in both pre- and post-treatment samplings. The very small differences in QBS-ar values between the two plots could be attributed to the absence of tractor passes during the Spintor® Fly application (August–September) in the DRO plot. In fact, the tractor never entered the DRO plot during this period, and previous research has demonstrated the high impact of soil compaction on QBS-ar values [31,37]. This may have contributed to an improvement in soil quality by reducing the effects of soil compaction caused by tractor passes. In this regard, the use of the drone represents a comparatively less impactful and more sustainable approach for the soil communities.

Similar outcomes were observed with the BLT. In fact, the CAP plots showed significantly higher bait consumption compared to the treated plots (CAS and DRO), and the consumption rates between the treated plots were not statistically different in both the pre- and post-treatment sampling periods. However, it is important to note that the feeding rate in the DRO plot was significantly higher than in the CAS plot during the post-treatment sampling. Previous studies have demonstrated that drones can provide an efficient and cost-effective solution for aerial spraying of pesticides in olive orchards [38]. In both QBS-ar and BLT, higher values were recorded in the post-treatment sampling compared to the pre-treatment sampling. These results could be attributed to the influence of climate. Indeed, the climatic conditions in July–August and the first half of September were much drier than those observed in the second half of September and October (Figure 2). More humid weather conditions had a significant effect on litter decay, primarily by influencing the abundance and activity of soil organisms [39].

Similar results were shown by the avoidance test, which recorded avoidance rates up to 60% in the comparison between control soil (CAP) and Casotti-treated soil (CAS) and a slightly lower rate in the comparison between control soil (CAP) and drone-treated soil (DRO). In any case, the results we obtained show avoidance rates below the 80% threshold, indicating a toxic environment [22]; similar results were also obtained by De Bernardi et al. (2022) [40] using natural soils artificially contaminated with different concentrations of spinosyns. In addition, the soil chemical parameters in the different plots were found to be extremely similar, and the differences in pre- and post-treatment were found to be constant.

Overall, all three methods used (i.e., QBS-ar, BLT and avoidance test) showed no statistically significant differences between the two Spintor® Fly-treated plots (DRO vs. CAS). Despite the lack of significant risk reduction effects, our results showed that there was not an increased risk when spraying PPPs with drones. According to this, drone spraying should be considered as a promising application strategy, hopefully to be included in European legislation. In fact, at similar levels of risk, the use of drones has important advantages over tractor application. Several studies confirm that drones provide greater precision (reducing time and PPP use), less soil compaction and better canopy distribution, thereby reducing the environmental impact of potential drift [12–15]. However, long-term studies are needed to highlight and validate the advantages of drone application and to further investigate the improvements in soil health, even if not significant, as we observed in this study.

Nevertheless, further research on drone spraying would be needed to highlight any beneficial effects associated with optimising PPP efficiency in relation to olive productivity.

Unfortunately, we were unable to measure the effects of the different application methods on olive productivity due to low yields caused by summer drought.

Additionally, an economic evaluation, to compare the costs of adopting drone spraying technology versus traditional methods, has been developed by a research group involved in the SFIDA project [21]. The analysis showed that the initial cost of the drone was slightly higher than that of the traditional method. Differences were identified in acquisition, maintenance, insurance costs, and personnel requirements between the two methods. Particularly, additional costs associated with drone technology—such as software systems for drone management and GPS—were highlighted. The solution proposed by the authors, to make this technology more accessible for smaller farms, is the combined purchase of drones. As the time required for spraying treatments is generally short, sharing drones among farms can ensure timely field interventions [21]. Overall, drone spraying represents a significant step towards digitization and implementation of innovative practises, such as precision farming.

Unfortunately, delays in obtaining the necessary permits for the aerial spraying of Spintor® Fly by drone, which in Italy is the responsibility of the Ministry of Health, have made it impossible to carry out a second monitoring season to better highlight and possibly consolidate the results obtained so far and to help promote the use of drones for spraying PPPs. Currently, the main restriction on the use of drones is related to potential environmental risk. We would like to emphasise that this was the first field study to assess that the drone-related risk was similar, if not better than, that associated with the traditional method. Therefore, concerns about environmental risk are, at worst, similar to current practises. We hope that the current Italian legislation will be updated to facilitate the granting of the necessary authorisations to verify the performance of drones in field spraying of PPPs, in order to increase the number of trials and the data available to assess the potential risks associated with their use in agriculture [15,20].

5. Conclusions

For the first time, the impact of two distinct methods of Spintor® Fly application—conventional (ground) and innovative (aerial)—on non-target soil organisms in an olive orchard was evaluated by studying soil communities in situ (QBS-ar and BLT) and ex situ by using an ecotoxicological test (avoidance test with earthworms). No significant differences were found between the two spraying methods used. However, drone aerial spraying showed slightly better performance, indicating a potentially lower impact on soil communities and soil health. The present results, together with previous research showing the advantages of drones over the traditional method in olive groves, provide clues for the use of drones as a sustainable solution in agriculture. Long-term studies are needed to assess the potential benefits of drone spraying over traditional methods and to confirm our results. These initial findings support that drone applications do not pose a higher environmental risk than traditional methods and should be considered in European Regulations to allow for further research, testing, and data collection.

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