

Article

Bioacoustics Reveal Species-Rich Avian Communities Exposed to Organophosphate Insecticides in Macadamia Orchards

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Simple Summary: Conventional commercial orchards often rely on chemical insecticides to control pests and diseases. Organophosphates are among the most widely used insecticides in agriculture. These insecticides are toxic to wildlife, and particularly to birds, which commonly forage for insects in orchards. In this study, we investigated the exposure of birds to a toxic organophosphate (trichlorfon) in Australian macadamia orchards. We used acoustic recorders to quantify bird activity and we used traps to measure insect abundance at six orchards, both before and after trichlorfon applications. Results indicated that the activity of all birds was similar before and after spraying and thus, the birds were exposed to the chemical. Furthermore, trichlorfon applications decreased the numbers of spiders at the orchards. We recorded a high diversity of bird species (62) in the orchards, and most of these birds (80%) were insectivores. Our results suggest that birds will continue foraging in treated orchards where they are exposed to insects contaminated with trichlorfon. We recommend that growers incorporate wildlife-friendly pest management strategies for macadamia orchards.

Abstract: Organophosphates are the most widely used insecticide class in agriculture. The effects of organophosphates on insectivorous birds can potentially reduce the capacity of these birds to regulate insect pest populations as well as jeopardizing the survival of vulnerable bird species in matrix habitats. In this study, we investigated the diversity of birds inhabiting commercial macadamia orchards in Australia and assessed community-wide exposure of birds to an organophosphate insecticide (trichlorfon). We also studied the impact of trichlorfon on arthropods, and how this affected bird activity. We used a novel approach, combining bird acoustic surveys, and three different arthropod trapping devices. Birds and arthropods were surveyed immediately before and after a trichlorfon application, in sprayed and unsprayed orchards, at six different sites. Surveys showed that trichlorfon applications produced no changes in bird activity, either at the species or community level. Only one species (*Lichmera indistincta*) showed a significant increase in acoustic activity after treatment. These results indicate that several (62) bird species, some of which have been noted as undergoing regional decline, are exposed to trichlorfon applications. Additionally, trichlorfon applications also produced rapid, negative impacts on certain arthropod groups, particularly spiders. Because almost (80%) of the bird species recorded in the study include arthropods in their diets, then arthropod contaminated by trichlorfon are likely consumed by these orchard-dwelling birds. We recommend that pest management should incorporate strategies to reduce wildlife exposure to

toxic chemicals to meet the joint goals of crop production and wildlife conservation in structurally complex agricultural habitats.

Keywords: bioacoustics; birds; arthropods; conservation; organophosphates; agriculture

1. Introduction

Globally, broad-spectrum insecticides are widely used in agriculture. Among these, organophosphates are the most frequently used insecticides [1,2]. These act primarily as inhibitors of the nervous system enzyme cholinesterase [3]. Several studies have reported an association between organophosphates and the mass death of wild birds [4,5]. Insecticides can impact farmland birds either directly, through lethal or sub-lethal poisoning, or indirectly, through a reduction in food resources [6].

Direct impacts on birds are produced through exposure to the insecticides via maternal transfer, inhalation, dermal contact, and/or the ingestion of insecticide contaminated food items such as insecticide-treated arthropods [7]. Among these, the latter is the main cause of insecticides poisoning among insectivorous birds [8]. A number of studies have shown how the ingestion of insects previously exposed to organophosphates can cause the death of birds (e.g., [9,10]). Additionally, cholinesterase-inhibiting insecticides can produce sub-lethal impacts, such as altering avian vocalization and reducing defense and territorial displays [11,12], which can ultimately lead to an abandonment of territories [12]. These behavioural responses to organophosphates may be short-lived, but if they produce the loss or abandonment of a breeding territory, they could impose delays in the breeding cycle and ultimately affect avian reproduction [13].

Insecticides can also indirectly impact avian reproduction and survival by reducing the abundance of food resources, such as invertebrates, [14,15] for insectivorous and omnivorous farmland birds [16]. Such indirect effects of insecticides may negatively impact numerous number of bird species [17,18]. The indirect effects of pesticide applications are difficult to measure because of the complexity of the phenomena that incorporates several trophic levels; however, such effects can be tested in the field. For example, in the United Kingdom, reductions of several farmland bird species have been associated with the indirect effects of insecticides [19] and reductions in invertebrate numbers after insecticide applications can result in birds moving to adjacent untreated areas for food [20,21], ultimately affecting avian reproduction [22].

A number of studies have examined the impact of insecticides on birds; however, for logistical reasons, most studies have only focused on one, or a few bird species (e.g., [11–16]), and have not examined the community wide impacts of insecticides applications. Acoustics sampling is a valuable tool that can reveal the spatial and temporal patterns of multiple bird species at the same time. Vocalizations offer a rich source of information on avian diversity, abundance and behaviour. In field surveys, birds are often more frequently heard than seen, and thus aural cues are increasingly used to index avian diversity and abundance [23]. Where bird species richness is high and/or when multiple skilled observers are unavailable, standardized acoustic surveys may be more feasible than ‘traditional’ bird surveys that are based on sightings (e.g., [24,25]). Furthermore, using automated acoustic recorders is a cost-effective way to increase the spatial and temporal scale of surveys, to reduce any inter-observer bias in data collection, and to reduce researcher impacts on wildlife activity [26]. Previous studies have used acoustic recorders to estimate the diversity and density of terrestrial birds (e.g., [23,25]), but to our knowledge, no previous study has used acoustic recorders to explore wildlife exposure to insecticides. In this study, acoustic recorders were used to monitor both individual species and community-wide bird activity, and point-counts were used to visually ratify the identity of some of some species. We conducted our study in the Bundaberg region, on the eastern coast of Queensland. This is an economically important agricultural region in Australia that consists of a diversity of crops interspersed among woodland patches. *Macadamia* (*Macadamia* spp.), a native Australian nut

producing tree, is one of the main crops produced in the region with a current cropping area of approximately 24,000 ha [27]. In the region, macadamia orchards are frequently located adjacent to forested natural habitats, and previous studies have shown that both the forests and the orchards play a key role in sustaining bird and bat communities [28,29]. Bird communities in macadamia orchards can be diverse, particularly those close to woodlands. These birds use the orchards as foraging habitat, where they can find water and food (i.e., invertebrate prey and nectar of macadamia flowers). However, a number of registered insecticides are used to control insect pests in macadamia orchards. Most of these insecticides have a broad-spectrum effect and contain either organophosphate compounds or synthetic pyrethroids [30]. Trichlorfon, an organophosphate insecticide used on a wide range of crops since the 1950s [3], is commonly applied to the region's macadamia orchards [30]. Although this insecticide has a short residual life [31], trichlorfon is considered moderately to highly toxic to birds [32]. A number of studies have shown that avian exposure to and/or ingestion of trichlorfon results in delayed neuropathy [33], reductions in brain cholinesterase activity [34], or dysbiosis in intestinal microbiota [35]. Hence, trichlorfon may impose a risk to avian communities, especially in structurally complex, high-diversity areas close to natural habitats.

In this study, we use a novel combination of acoustic surveys and arthropod survey methods to: (i) examine the diversity of avian communities occurring in macadamia orchards, (ii) assess bird responses at both the species and community levels to trichlorfon applications by measuring bird acoustic activity before and immediately after insecticide applications, (iii) assess the impact of insecticide applications on arthropod abundance in the orchards, and (iv) to determine whether changes in the arthropod community following trichlorfon applications are associated with bird activity.

2. Experimental Section

2.1. Study Area

The study was conducted in the Bundaberg region (Lat. 25.096° S, Lon. 152.334° E), which has a subtropical climate on the coast of eastern Australia (Figure 1). Agricultural development in this region has resulted in a fragmented landscape with a mix of isolated and interconnected dry and sclerophyll remnant woodland patches. All surveyed sites were located in areas of macadamia production spread throughout the region. The sites were located on alluvial red soils and were adjacent to woodland areas of similar vegetation composition. The dominant tree species in these woodlands were the forest red gum (*Eucalyptus tereticornis*), black wattle (*Acacia leiocalyx*), bloodwood (*Corymbia intermedia/trachyphloia*), swamp mahogany (*Lophostemon suaveolens*) and tea tree species (*Melaleuca* spp.). Woodland understories varied according to management history, but generally consisted of grasses and various woody shrubs, especially *Acacia* species. The Bundaberg region has a summer dominant rainfall and high temperatures with average annual rainfalls of 1015 mm and a maximum average temperature of 26.7 °C based on a climatic standard of 63 years (1942–2020) and 42 years (1959–2020) respectively [36].

2.2. Experimental Design

Six locations in the Bundaberg region, each consisting of commercial macadamia orchards, were selected for this study (Figure 1). These macadamia orchards shared similar characteristics: adult trees (>7 years old) with a canopy height of between 3 and 5 m, and with a mixture of commercial cultivars that typically included cv '344' and cv '600'. Each of the six locations were at least 1000 m apart (Figure 1). All locations followed similar conventional crop management practices, including the application of insecticides with air blast sprayers to reduce pest pressures.

To study the short-term impacts of trichlorfon applications on bird and arthropod communities, a paired-sites design was used: at each location, two sites were assigned as either recently sprayed (henceforth 'sprayed') or not sprayed for at least 45 days (henceforth 'unsprayed', control sites). Each pair of sites shared similar environmental conditions (i.e., vegetation structure, soil type, macadamia variety).

All pairs of sites were located at a fixed distance of 100 m from woodland. The average distance between 'sprayed' and 'unsprayed' sites was 275 m (minimum = 250 m, maximum = 320 m) (Figure 1).

During the study, the commercial organophosphate insecticide Lepidex 500[®] (containing 500 g/L trichlorfon) (see detailed information on Appendix A) was applied at the recommended dose of 200 mL/100 L only at the 'sprayed' sites. Water-sensitive spray cards were used at each site to ensure that 'unsprayed' sites were not affected by insecticide drift. Surveys were simultaneously performed in each pair of sites at each location during three different times. These were: one day before the insecticide was applied (hereafter 'pre'), and one and three days afterwards (hereafter 'post 1' and 'post 3', respectively). These sampling times were used as a three-level factor in subsequent analyses. All surveys were undertaken in 2014, during the last two weeks of September, overlapping with the breeding season for most bird species in the region, between August and January. Additionally, during this period the macadamia phenology was between the peak of flowering and early nut development phases. At this time, the numbers of insect herbivores normally increase on the crop [29], so insecticide treatments are generally conducted in the orchards.

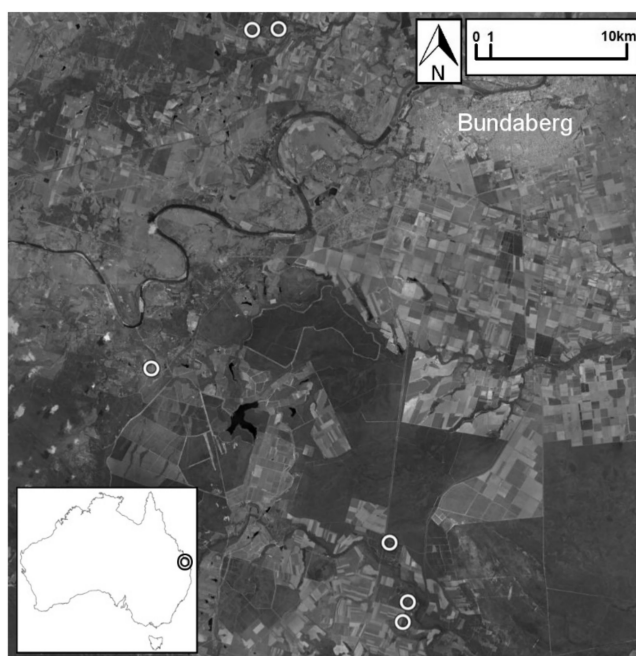


Figure 1. Map showing the geographic location of the studied area and the surveyed locations, represented as circles.

2.3. Acoustic Surveys

Bird vocalizations were used to estimate species diversity and comparative abundance. Two acoustic recorders (Song Meter SM2, Wildlife Acoustics) were programmed to record at 44,100 Hz simultaneously at each location, one at each site. Avian acoustic activity was continuously recorded for three hours at each site, starting at 30 min before sunrise. Each Song Meter was set-up at the top of a 3 m vertical pole. Concurrently with each acoustic survey, 30 min after sunrise, a ten-minute point count was conducted at each location, preceded by a 3-min 'relaxation period'. Because all locations were macadamia orchards, there was often activity by workers that occasionally included the operation of machinery, thus hides were not used during the surveys. Surveys were carried out from high ground from which the entire location (including sprayed and unsprayed sites) could be scanned using binoculars. To avoid bias, all point counts were carried out by the same observer (EC), and only birds that were directly observed at each site were noted. Birds overflying the sites were not noted. Weather conditions remained stable (i.e., no rain or heavy winds) during the surveys, which improved our ability to compare results between sites and sampling periods.

To reduce the time for analysing the acoustic dataset, while retaining a high level of species detection accuracy, we followed the methodology of Wimmer et al. [37] to sample avian communities along acoustic recordings. Thirty one-minute segments were randomly selected from the first three recorded hours per site, and analysed manually to identify all bird species and quantify their acoustic activity by means of counting calls and/or songs. In this study, avian acoustic activity represents a measure of relative abundance between times, thus it serves as a proxy indicator of bird activity and is not absolute. Based on a number of reference materials (i.e., [38–40]) and the Xeno-Canto online database (<http://www.xeno-canto.org>), a library was created which included the most common calls and/or songs for each of the bird species recorded. Each one-minute segment was played and visually scanned by EC using audio editor software (Adobe Audition CS6, version 5.0.2), and the calls and songs found were manually assigned to each species. Species assignments were ratified by Frederik Willem Van Gessel and Anthony Baylis, both experts in bird acoustic identification, from the Australian Wildlife Sound Recording Group (<https://awsrg.org.au/>). Furthermore, each bird species was assigned a priori to a functional guild based on its primary foraging habitat and diet preferences (i.e., based on [38–40]).

2.4. Arthropod Surveys

Three arthropod trapping devices were used to sample each location: these were sweep-nets, light-traps and pit-fall traps. Sweep-net sampling was conducted to estimate the arthropod community at the tree-canopy level, i.e., at a height of 1 to 4 m across the macadamia canopy. Nets of 38 cm diameter were used to sample the two locations (sprayed and unsprayed) at each site, immediately after acoustic surveys were completed. Each of the sweep-net samples consisted of a total of 120 sweeps consisting of four random sub-samples each with 30 sweeps along a 25 m transect. All sampling was conducted following the same transect at each location, all within 50 m from the Song Meter. Captures from each sample were immediately placed in sealed plastic bags containing an acetone-soaked cotton ball to kill the arthropods. For each sample, captures from the sweep nets were pooled. Light trap sampling was conducted to characterise nocturnal arthropod communities. One light trap (12 V, 8 W) was set-up at each location and programmed using timers to operate for 3 h with nightly sampling ceasing at 4 h before sunrise. Before the start of acoustic surveys, all arthropod captures from each light trap were collected into plastic vials containing 70% ethanol. Pit-fall traps were used to estimate the relative abundance of ground-dwelling arthropods. Three cylindrical pit-fall traps, each of 10 cm in height and 7 cm in diameter were spaced at 10 m intervals along a transect. The traps were opened at sunset and collected after 24 h. At each location, the contents of the three pit-fall traps were pooled and placed into a single plastic vial filled with 70% ethanol.

All collected arthropods were identified to Order under a dissecting microscope (Olympus SZ51) following Zborowski and Storey [41]. Order was chosen as a suitable taxonomic level that allows rapid and reliable biodiversity assessments in agricultural systems [42]. A number of arthropod taxa constituted exceptions to the order level: all mites were classified as Subclass Acari. Hymenoptera was split into Family Formicidae (i.e., ants) and 'other' Hymenoptera (i.e., bees and wasps). After identification, arthropod samples were placed in an oven at 60 °C for 48 h. The total dry weight of each sample was then measured to the nearest 0.1 mg using an electronic balance.

2.5. Statistical Analyses

One-way analysis of variance (ANOVA) was used to examine the abundance and dry weight of arthropods, the frequency of vocalisations for each bird species, and the abundance of each bird species based on 10 min point counts. Given the natural wide variation in diversity and abundance of birds and arthropods across locations, ratios were calculated using the values of each of the aforementioned variables at each location (i.e., 'sprayed' site/['unsprayed' site + 'sprayed' site]). Ratios had values between 0 and 1, with 1 indicating no activity or abundance recorded at unsprayed sites. These ratios were subjected to ANOVA, allowing us to test for differences in abundance between pre- and post-insecticide

application samples. Data was checked to ensure that it satisfied the assumptions of the ANOVA, and data transformations were applied when necessary. Whenever a significant result was found, Holm-Bonferroni sequential correction tests were calculated to differentiate between levels.

Permutational multivariate analysis of variance (PERMANOVA) [43] was used to test for differences across factors in the structure of arthropod and bird communities. For the analysis of bird communities, all species were pooled together, regardless of their guilds, since ca. 80% of all bird species recorded were fully or partially insectivorous, and previous research has shown that 'non insectivorous' species can occasionally feed also on insects [28]. 'Location' was treated as a random factor with six levels (one per location), and 'Time' (with three levels: 'pre', 'post 1', and 'post 3') and 'Spray' (with two levels: 'sprayed' and 'unsprayed') were considered fixed factors. The 'Time × Spray' interaction term allowed us to detect changes at the community level by contrasting the effects across time at both 'sprayed' and 'unsprayed' sites. Whenever significant results were found, PERMANOVA pair-wise tests were used to check for differences between levels. PERMANOVA analyses were based on Bray-Curtis similarity resemblance matrices of log-transformed data (avian acoustic activity dataset) and root square-transformed data (arthropod dataset), each analysis permuted 9999 times. Permutational analysis of multivariate dispersions (PERMDISP) was used to test the homogeneity of data dispersion by calculating the distance to group centroids [44]. Non-metrical multidimensional scaling (nMDS) was used to visually compare avian acoustic activity across factors.

The ANOVA analyses were performed on SPSS (v. 22). PERMANOVA, PERMDISP and nMDS routines were performed with PRIMER (v. 6.1.16) and the PERMANOVA + extension (v. 1.0.6).

3. Results

3.1. Avian Acoustic Activity

Overall, 60,680 calls and songs were positively identified and assigned to a total of 62 bird species during the study (Table 1). Of these species, 50 (80.6%) are considered either fully or partially insectivorous [38–40]. Nineteen species (30.6%) were recorded across all the locations during the study. Seven species recorded in this study are considered 'decliners' (i.e., species consistently declining in abundance over the last decade) in eastern Queensland [45] (Table 1). Of the 62 species recorded with acoustic recorders, 47 (75.8%) were also observed during the 10 min point counts (Table 1). Analysis of acoustic activity ratios at the species level showed only one species, *Lichmera indistincta*, with significantly different values between times ($F = 4.082$, $p = 0.042$); the species appeared more active in the 'post 3' than in the 'pre' sample (Table 1). None of the species observed during 10 min point counts showed differences in abundance between times ($p > 0.05$) (Table S1).

Table 1. Avian species recorded with acoustic recorders during the study, with assigned guild, the number of locations in which each species was found, the contribution of individual species calls and/or songs as a percentage of the total recorded, and individual averaged acoustic activity ratio across time (average \pm SE).

Common Name	Scientific Name	Guild ¹	Loc ²	% ³ Calls ³	Acoustic Activity Ratios (Average \pm SE) ⁴		
					Pre ⁵	Post 1	Post 3
Silvereye ⁷	<i>Zosterops lateralis</i>	Sh-omni	6	38.04	0.50 \pm 0.12	0.44 \pm 0.20	0.44 \pm 0.15
Bar-shouldered Dove ⁷	<i>Geopelia humeralis</i>	Gr-grani	6	6.94	0.33 \pm 0.12	0.60 \pm 0.20	0.35 \pm 0.23
Rufous Whistler ⁷	<i>Pachycephala rufiventris</i>	Sh-insect	6	6.88	0.46 \pm 0.23	0.45 \pm 0.18	0.57 \pm 0.25
Little Friarbird ⁷	<i>Philemon citreogularis</i>	Ca-nectinsect	6	6.68	0.41 \pm 0.10	0.43 \pm 0.12	0.47 \pm 0.25
Noisy Friarbird ⁷	<i>Philemon corniculatus</i>	Ca-omni	6	4.60	0.46 \pm 0.45	0.52 \pm 0.43	0.42 \pm 0.37
Torresian Crow ⁷	<i>Corvus orru</i>	Gr-omni	6	4.48	0.44 \pm 0.32	0.66 \pm 0.22	0.53 \pm 0.25
Little Shrike-thrush ⁷	<i>Colluricincla megarhyncha</i>	Ca-insect	6	2.73	0.55 \pm 0.12	0.43 \pm 0.15	0.50 \pm 0.16
Olive-backed Oriole ⁷	<i>Oriolus sagittatus</i>	Ca-nectinsect	6	1.94	0.65 \pm 0.22	0.51 \pm 0.17	0.46 \pm 0.33
Pied Butcherbird ⁷	<i>Cracticus nigrogularis</i>	Ge-omni	6	1.82	0.54 \pm 0.19	0.45 \pm 0.14	0.57 \pm 0.14
White-browed Scrubwren	<i>Sericornis frontalis</i>	Sh-insect	6	1.76	0.54 \pm 0.35	0.56 \pm 0.38	0.58 \pm 0.33
Brown Honeyeater ^{6,7}	<i>Lichmera indistincta</i>	Sh-nectinsect	6	1.30	0.20 \pm 0.21 a	0.47 \pm 0.24 ab	0.66 \pm 0.27 b
Willie Wagtail ⁷	<i>Rhipidura leucophrys</i>	Sh-insect	6	1.27	0.35 \pm 0.39	0.69 \pm 0.37	0.30 \pm 0.31
Dusky Honeyeater	<i>Myzomela obscura</i>	Ca-nectinsect	6	1.26	0.52 \pm 0.45	0.22 \pm 0.38	0.25 \pm 0.34
Eastern Yellow Robin ⁷	<i>Eopsaltria australis</i>	Gr-insect	6	1.16	0.68 \pm 0.33	0.53 \pm 0.29	0.67 \pm 0.29
Magpie-lark ⁷	<i>Grallina cyanoleuca</i>	Gr-insect	6	1.11	0.47 \pm 0.26	0.46 \pm 0.28	0.58 \pm 0.25
Grey Butcherbird ⁷	<i>Cracticus torquatus</i>	Ca-omni	6	0.94	0.51 \pm 0.26	0.44 \pm 0.26	0.73 \pm 0.22
Australian Magpie ⁷	<i>Gymnorhina tibicen</i>	Gr-insect	6	0.60	0.37 \pm 0.34	0.59 \pm 0.24	0.37 \pm 0.20
Lewin's Honeyeater ⁷	<i>Meliphaga lewinii</i>	Ca-nectinsect	6	0.31	0.45 \pm 0.38	0.24 \pm 0.28	0.62 \pm 0.34
Grey Shrike-thrush ⁷	<i>Colluricincla harmonica</i>	Ge-insect	6	0.30	0.64 \pm 0.37	0.55 \pm 0.34	0.57 \pm 0.39
Eastern Koel ⁷	<i>Eudynamys orientalis</i>	Ca-frugi	5	2.16	0.58 \pm 0.30	0.35 \pm 0.22	0.64 \pm 0.21
Fairy Gerygone ⁷	<i>Gerygone palpebrosa</i>	Ca-insect	5	0.70	1.00 \pm 0.00	0.42 \pm 0.42	0.43 \pm 0.47
White-winged Chough ⁷	<i>Corcorax melanorhamphos</i>	Gr-omni	5	0.48	0.55 \pm 0.41	0.86 \pm 0.21	0.51 \pm 0.16
Black-faced Cuckoo-shrike ^{6,7}	<i>Coracina novaehollandiae</i>	Ca-insect	5	0.25	0.25 \pm 0.20	0.39 \pm 0.37	0.61 \pm 0.29
Eastern Rosella ⁷	<i>Platycercus eximius</i>	Gr-grani	5	0.19	0.67 \pm 0.00	0.25 \pm 0.43	0.90 \pm 0.15
Brush Cuckoo	<i>Cacomantis variolosus</i>	Sh-insect	5	0.17	1.00 \pm 0.00	0.74 \pm 0.24	0.69 \pm 0.41
Little Wattlebird	<i>Anthochaera chrysoptera</i>	Sh-nectinsect	5	0.17	1.00 \pm 0.00	0.33 \pm 0.47	0.96 \pm 0.04
Laughing Kookaburra ^{6,7}	<i>Dacelo novaeguineae</i>	Gr-carni	5	0.08	0.59 \pm 0.07	0.16 \pm 0.20	0.65 \pm 0.15
Purple Swampphen ⁷	<i>Porphyrio porphyrio</i>	Aq-omni	5	0.06	0.49 \pm 0.33	0.50 \pm 0.50	0.33 \pm 0.47
Noisy Miner ⁷	<i>Manorina melanocephala</i>	Ca-nectinsect	4	1.79	0.88 \pm 0.13	0.66 \pm 0.47	0.47 \pm 0.47
Pheasant Coucal ⁷	<i>Centropus phasianinus</i>	Gr-carni	4	0.24	0.66 \pm 0.36	0.21 \pm 0.21	0.60 \pm 0.12
Rainbow Lorikeet ⁷	<i>Trichoglossus moluccanus</i>	Ca-nectinsect	4	0.23	0.64 \pm 0.35	0.45 \pm 0.32	0.11 \pm 0.11
White-throated Treecreeper ⁷	<i>Cormobates leucophaea</i>	Ca-insect	4	0.22	1.00 \pm 0.00	0.42 \pm 0.00	0.65 \pm 0.46

Table 1. Cont.

Common Name	Scientific Name	Guild ¹	Loc ²	% ³ Calls ³	Acoustic Activity Ratios (Average ± SE) ⁴		
					Pre ⁵	Post 1	Post 3
Sulphur-crested Cockatoo ⁷	<i>Cacatua galerita</i>	Ge-grani	4	0.20	0.88 ± 0.00	0.50 ± 0.50	0.39 ± 0.39
Double-barred Finch ^{6,7}	<i>Taeniopygia bichenovii</i>	Sh-grani	4	0.09	0.00 ± 0.00	-	0.50 ± 0.50
Black Cockatoo ⁷	<i>Calyptorhynchus banksii</i>	Ca-frugi	3	1.65	0.66 ± 0.00	0.99 ± 0.01	0.87 ± 0.19
Peaceful Dove ^{6,7}	<i>Geopelia placida</i>	Gr-grani	3	0.89	0.00 ± 0.00	0.28 ± 0.33	0.18 ± 0.00
Channel-billed Cuckoo ⁷	<i>Scythrops novaehollandiae</i>	Ca-frugi	3	0.60	-	0.00 ± 0.00	0.57 ± 0.10
Blue-faced Honeyeater	<i>Entomyzon cyanotis</i>	Ca-nectinsect	3	0.37	0.48 ± 0.41	0.11 ± 0.04	0.32 ± 0.27
White-throated Gerygone ⁷	<i>Gerygone olivacea</i>	Ca-insect	3	0.13	1.00 ± 0.00	1.00 ± 0.00	0.94 ± 0.00
Cicadabird	<i>Coracina tenuirostris</i>	Ca-insect	3	0.12	0.00 ± 0.00	0.50 ± 0.50	0.50 ± 0.50
Rainbow Bee-eater ^{6,7}	<i>Merops ornatus</i>	Ae-insect	3	0.11	0.78 ± 0.22	1.00 ± 0.00	1.00 ± 0.00
Fan-tailed Cuckoo ⁷	<i>Cacomantis flabelliformis</i>	Sh-insect	3	0.04	0.50 ± 0.50	1.00 ± 0.00	0.73 ± 0.03
Pied Currawong ⁷	<i>Strepera graculina</i>	Sh-omni	3	0.03	1.00 ± 0.00	-	1.00 ± 0.00
Sacred Kingfisher	<i>Todiramphus sanctus</i>	Gr-carni	3	0.02	0.00 ± 0.00	1.00 ± 0.00	0.00 ± 0.00
Leaden Flycatcher ⁷	<i>Myiagra rubecula</i>	Sh-insect	2	0.64	0.39 ± 0.00	0.54 ± 0.00	0.72 ± 0.28
Dusky Moorhen ⁷	<i>Gallinula tenebrosa</i>	Aq-omni	2	0.40	0.00 ± 0.00	0.42 ± 0.25	0.27 ± 0.27
Masked Lapwing ⁷	<i>Vanellus miles</i>	Gr-insect	2	0.19	0.50 ± 0.50	0.15 ± 0.15	-
Wonga Pigeon	<i>Leucosarcia melanoleuca</i>	Gr-grani	2	0.08	0.50 ± 0.50	1.00 ± 0.00	-
Spangled Drongo ^{6,7}	<i>Dicrurus bracteatus</i>	Ca-omni	2	0.07	-	0.00 ± 0.00	0.36 ± 0.00
Red-browed Finch	<i>Neochmia temporalis</i>	Sh-grani	2	0.06	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Dollarbird ⁷	<i>Eurystomus orientalis</i>	Ae-insect	2	0.04	-	1.00 ± 0.00	0.00 ± 0.00
Varied Triller ⁷	<i>Lalage leucomela</i>	Ca-omni	2	0.03	0.50 ± 0.50	1.00 ± 0.00	1.00 ± 0.00
Galah ⁷	<i>Eolophus roseicapilla</i>	Gr-grani	2	0.03	-	0.69 ± 0.00	1.00 ± 0.00
White-breasted Woodswallow	<i>Artamus leucorhynchus</i>	Ae-insect	2	0.01	0.00 ± 0.00	0.30 ± 0.30	-
Mistletoe	<i>Dicaeum hirundinaceum</i>	Ca-frugi	2	0.01	1.00 ± 0.00	-	1.00 ± 0.00

¹' indicates guild, composed by two terms, the first one showing the most frequent foraging habitat (Gr: ground, Sh: shrubs, Ca: tree canopy, G: generalist, Aq: aquatic), and the second one showing the diet (insect: insectivore, nectar: nectarivore, nectinsect: partially nectarivore and insectivore, grani: granivore, frugi: frugivore, omni: omnivore, and carni: carnivore). ²' indicates the number of locations in which the species was recorded. ³' indicates the proportion of all calls and songs recorded from an individual species over the total. ⁴' Ratios were calculated using the acoustic activity of each species at each location (i.e., 'sprayed' site/['unsprayed' site + 'sprayed' site]). ⁵' Times when surveys were conducted: one day before the insecticide was applied ('pre'), and one ('post 1') and three days afterwards ('post 3'). ⁶' indicates that this species has shown consistent declines in numbers in eastern Queensland over the last 10 years (Birdlife, 2015). ⁷' indicates that this species was observed during the 10 min point count. Groups with the same letter are not significantly different based on Holm-Bonferroni sequential correction test results. Seven bird species were recorded at only one location with acoustic recorders and were not included in this table. These species were: Australian Figbird ⁷ (*Sphecothebes vieilloti*), Striated Pardalote (*Pardalotus striatus*), Eastern Whipbird (*Psophodes olivaceus*), Jacky Winter (*Microeca fascinans*), Golden Whistler ⁷ (*Pachycephala pectoralis*), Pacific Black Duck ⁵ (*Anas superciliosa*), and White-plumed Honeyeater (*Ptilotula penicillata*).

At the community level, PERMANOVA results showed that ‘Location’ had a highly significant impact on the acoustic activity of birds (Table 2, Figure 2). Two interaction terms, ‘Spray × Location’ and ‘Time × Location’, were highly significant, in contrast to the ‘Time × Spray’ term, which was non-significant (Table 2, Figure 2). When the dataset was transformed to presence-absence of species, results were highly similar to those aforementioned (Table 2). PERMDISP tests showed that PERMANOVA results were not affected by deviations from the centroid in any of the three factors ($p > 0.05$). Additionally, no significant variations were found in the acoustic activity of any of the avian guilds ($p > 0.05$), thus these results will not be discussed further.

Table 2. PERMANOVA Pseudo-F values examining the effect of time, spray application and location on arthropods captured with three different trapping devices, and on avian acoustic activity.

Source of Variation	df	Arthropod Abundance by Sampling Method			Avian Acoustic Activity	
		Light-Traps	Pit-Fall Traps	Sweep-Nets	Log-Transformed Data	Presence/abs. Data
Time	2	1.970	2.413 *	1.346	1.038	1.087
Spray	1	0.721	1.597	1.125	0.704	0.805
Location (Loc)	5	4.636 ***	5.695 ***	14.319 ***	22.680 ***	15.503 ***
Time × Spray	2	1.079	2.087 *	2.167 *	1.492	1.651
Time × Loc	10	1.879 *	1.260	1.594	2.802 ***	2.074 ***
Spray × Loc	5	1.930 *	2.686 ***	3.101 **	3.423 ***	2.346 ***

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

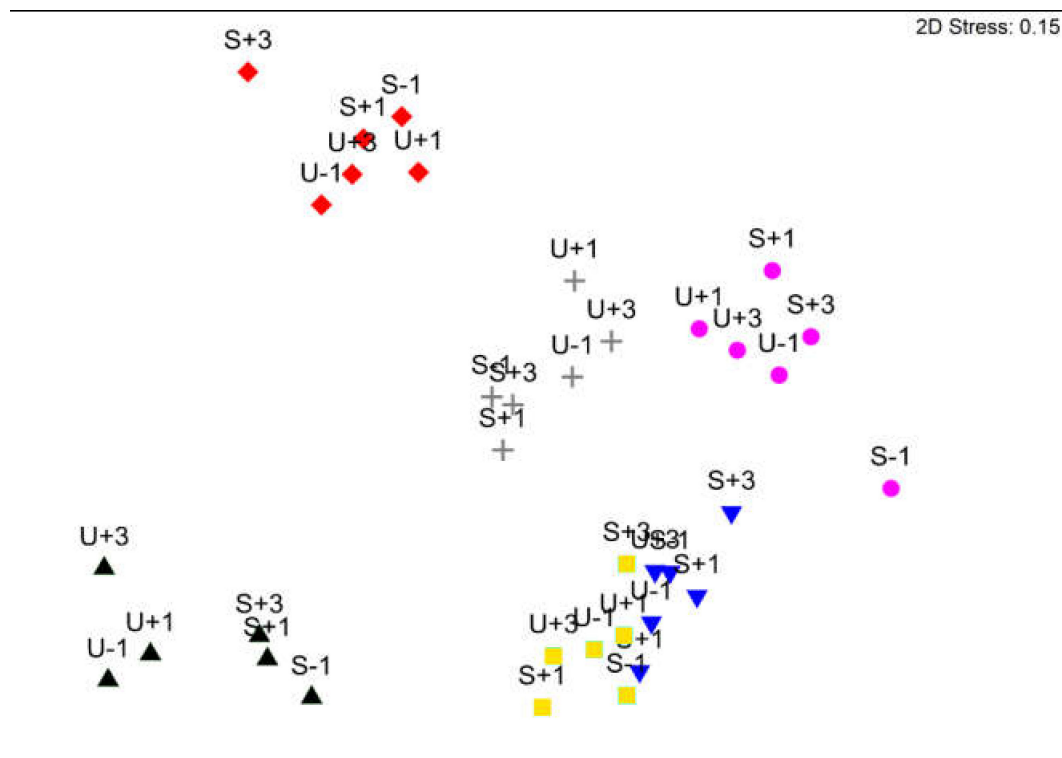


Figure 2. Non-metric multidimensional scaling (nMDS) ordination of avian acoustic activity across all factors. Different symbols indicate different locations. Over each symbol, ‘U’ or ‘S’ indicates an ‘unsprayed’ or ‘sprayed’ site, respectively, followed by ‘-1’, ‘+1’ or ‘+3’, which indicate ‘pre’, ‘post 1’, or ‘post 3’ groups, respectively. The latter groups indicate the times when surveys were conducted: one day before the insecticide was applied (‘pre’), and one (‘post 1’) and three days afterwards (‘post 3’).

3.2. Arthropods

Over the course of the experiment, we captured and identified 38,168 arthropods. Of these, 33,039 (86.56% of all arthropods) were captured using sweep-nets, 3140 (8.23%) were captured using pit-falls and 1989 (5.21%) were capture with light-traps. Arthropod abundance ratios did not significantly change across sampling times in any of the three arthropod trapping devices (Figure 3A). There was a decline in canopy arthropod dry weights according to sweep-net samples, after the insecticide applications ($F = 5.658, p = 0.019$) (Figure 3A).

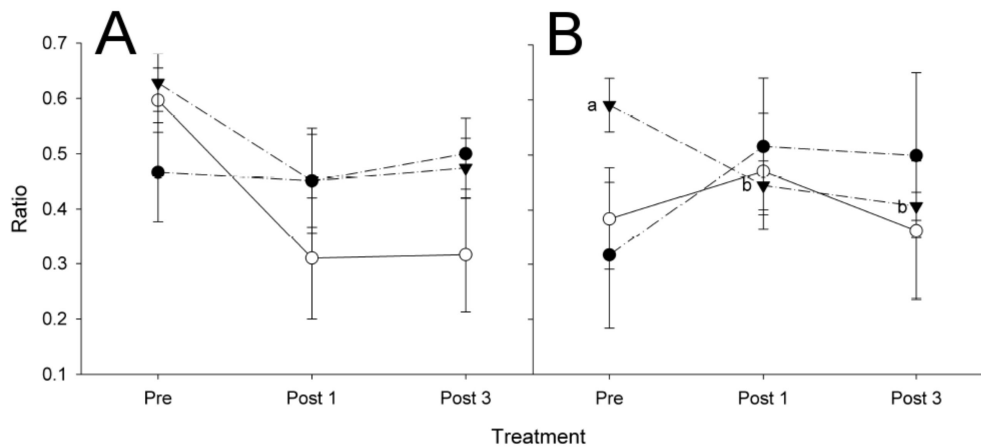


Figure 3. Between-time differences in arthropod abundance (A) and total dry-weight (B) per trapping device expressed as averaged ratios (\pm SE). Ratios were calculated using the abundance and total dry weight of each taxon at each location (i.e., ‘sprayed’ site/[‘unsprayed’ site + ‘sprayed’ site]). Times when surveys were conducted are indicated: one day before the insecticide was applied (‘pre’), and one (‘post 1’) and three days afterwards (‘post 3’). Black circles represent light-trap samples, white circles represent pit-fall samples and inverted black triangles represent sweep-net samples. Different letters represent significant differences between times based on Holm-Bonferroni sequential correction test results.

PERMANOVA results showed that both ‘Location’ and ‘Spray \times Location’ had significant effects on the structure of the arthropod community across all trapping devices (Table 2). Arthropods collected using sweep-net and pit-fall traps also showed a significant ‘Time \times Spray’ interaction ($p > 0.05$); sweep-net samples showed significant pair-wise differences in the structure of the arthropod community between ‘pre’ and ‘post1’ samples at ‘sprayed’ sites ($p < 0.05$), whereas pitfall samples showed differences between ‘pre’ and ‘post3’ samples on ‘sprayed’ sites ($p < 0.05$). No differences were found in any of the latter trapping devices on ‘unsprayed’ sites ($p > 0.05$). PERMDISP results showed that PERMANOVA results were not affected by data spread in any of the three factors ($p > 0.05$).

ANOVA tests showed that the abundance ratios of Araneae (spiders) captured with sweep-nets were significantly lower in the ‘post3’ than in the ‘pre’ samples ($F = 3.679, p = 0.050$) (Table 3). Spiders captured with pit-falls also decreased from ‘pre’ to ‘post 3’ samples, but in this case differences were not significant ($p > 0.05$) (Table 3). No other significant differences within the ‘Time’ factor were found in any other arthropod taxa using any of the trapping methods.

Table 3. Arthropod taxa recorded per trapping device during the study, with total abundance, and individual averaged abundance ratio across time (average \pm SE).

Trap	Taxon ¹	Abundance	Abundance Ratios (Average \pm SE) ²		
			Pre ³	Post 1	Post 3
Sweep-net	Hemiptera		0.68 \pm 0.08	0.42 \pm 0.14	0.56 \pm 0.10
Sweep-net	Coleoptera	7123	0.57 \pm 0.08	0.52 \pm 0.12	0.54 \pm 0.11
Sweep-net	Diptera	3205	0.54 \pm 0.05	0.43 \pm 0.09	0.52 \pm 0.04
Sweep-net	Hymenoptera	1921	0.58 \pm 0.06	0.43 \pm 0.07	0.50 \pm 0.05
Sweep-net	Araneae	1488	0.53 \pm 0.05 a	0.40 \pm 0.06 b	0.35 \pm 0.03 b
Sweep-net	Psocoptera	993	0.57 \pm 0.08	0.51 \pm 0.05	0.48 \pm 0.09
Sweep-net	Formicidae	694	0.58 \pm 0.06	0.45 \pm 0.11	0.38 \pm 0.05
Sweep-net	Lepidoptera	503	0.69 \pm 0.14	0.39 \pm 0.15	0.29 \pm 0.14
Sweep-net	Neuroptera	413	0.52 \pm 0.07	0.54 \pm 0.09	0.45 \pm 0.05
Sweep-net	Thysanoptera	205	0.69 \pm 0.11	0.39 \pm 0.13	0.30 \pm 0.12
Sweep-net	Orthoptera	50	0.70 \pm 0.20	0.75 \pm 0.16	0.53 \pm 0.22
Pit-fall	Formicidae	1932	0.60 \pm 0.08	0.35 \pm 0.15	0.52 \pm 0.12
Pit-fall	Collembola	923	0.40 \pm 0.13	0.20 \pm 0.12	0.25 \pm 0.13
Pit-fall	Diptera	86	0.47 \pm 0.16	0.49 \pm 0.15	0.23 \pm 0.15
Pit-fall	Coleoptera	83	0.63 \pm 0.14	0.78 \pm 0.14	0.50 \pm 0.29
Pit-fall	Araneae	61	1.00 \pm 0.35	0.47 \pm 0.23	0.43 \pm 0.16
Light-trap	Lepidoptera	1198	0.46 \pm 0.16	0.43 \pm 0.12	0.53 \pm 0.11
Light-trap	Coleoptera	337	0.57 \pm 0.10	0.45 \pm 0.15	0.45 \pm 0.19
Light-trap	Diptera	315	0.55 \pm 0.18	0.60 \pm 0.11	0.50 \pm 0.07
Light-trap	Hymenoptera	57	0.53 \pm 0.08	0.50 \pm 0.16	0.29 \pm 0.20
Light-trap	Hemiptera	51	0.50 \pm 0.50	0.58 \pm 0.30	0.60 \pm 0.40

Different letters indicate different groups based on Holm-Bonferroni sequential correction test results. ¹: please note that only taxa with abundance equal to or greater than 50 individuals were included. ²: Ratios were calculated using the abundance of each taxon at each location (i.e., 'sprayed' site/'unsprayed' site + 'sprayed' site). ³: Times when surveys were conducted: one day before the insecticide was applied ('pre'), and one ('post 1') and three days afterwards ('post 3').

4. Discussion

Using a novel combination of bioacoustics, point-counts and arthropod survey methods, this study presents evidence of community-wide exposure of birds to trichlorfon applications in macadamia orchards. Trichlorfon produced negative effects on non-target organisms by reducing the numbers and biomass of non-target arthropods, particularly spiders. Changes in the abundance of arthropods, without apparent behavioural changes in insectivorous birds following trichlorfon applications suggests that birds may be prone to ingest arthropod food items (either dead, dying, or with sub-lethal doses) contaminated with the organophosphate insecticide. The birds were also prone to dermal and respiratory uptake of the toxic chemical.

4.1. The Importance of Macadamia Landscapes as Habitat for Birds and Arthropods

This study also indicates the habitat value of macadamia orchards and nearby eucalyptus woodland patches for wild birds and the importance of arthropods in driving bird species richness. Overall, 62 bird species were recorded in this study, though only 19 species were common across all locations. Indeed, location had a significant influence on the structure of both the avian and the arthropod communities, an indication that the presence of these animals in agricultural areas is determined by characteristics of the adjacent natural habitats, as other studies have shown before (e.g., [46,47]). These results also support those conducted earlier in macadamia landscapes in the Bundaberg area: Crisol-Martínez et al., captured 11 bird species using mist-nets in a macadamia orchard located adjacent to a forested habitat [28]. Most of these birds were insectivorous and fed on macadamia pests, thus their foraging activities directly translated into pest-regulation services. In the present study, despite using different survey methods, we were able to record not only those same 11 species from the previous study, but several more from the macadamia sites. Additionally, 10 min

point counts found 47 out of the 62 species recorded with acoustic recorders, which indicates that acoustic recorders can be more efficient as they require a lower survey effort and detect birds that may be hidden by branches or foliage in the structurally complex macadamia orchards [24,25]. Besides birds and arthropods, the mixed forest-orchard landscapes in Bundaberg maintain rich insectivorous bat communities, which are also highly active in macadamia stands [29]. A study of macadamia in South Africa has also indicated the importance of macadamia landscapes as habitat for birds, as well as bats and monkeys [48].

4.2. Bird Exposure to Organophosphates

Our results strongly suggest that avian communities foraging in macadamia orchards are exposed to trichlorfon: we found bird acoustic activity was similar before and after insecticide applications. Acoustic surveys indicated that the structure of the avian communities at the macadamia stands were unaffected by the chemical applications. A lack of significance in the analysis when applying presence-absence transformations over the same data demonstrated that inter-species differences in vocalization (i.e., number of calls and/or songs per unit of time) did not bias the results. When birds were studied individually, only one species (*L. indistincta*) showed differences in its acoustic activity across time, however, contrary to what might be predicted, its activity gradually increased after insecticide spraying. Overall, these results do not support those found by Bouvier et al., who suggested that bird communities foraging in apple orchards respond quickly to pesticide applications, showing reductions in abundance and diversity [49]. Previous studies investigating broad-spectrum insecticides (other than trichlorfon) in rangelands have also reported conflicting results. George et al. observed similar levels of bird species diversity and richness before and after spraying [50], whereas Norelius and Lockwood found population reductions of most bird species after sites were sprayed [51]. To our knowledge, only one study, conducted in Europe, has investigated the impact of an organophosphate insecticide (chlorpyrifos) on avian communities. In this telemetry-based study, researchers found that 80% of individually monitored birds remained in sprayed crops (most of them orchard systems) during insecticide applications [52]. Our study, which is the first to assess an avian community response to pesticides in Australia, agrees with the previous study, by indicating that a large number of Australian bird species, including seven species that are declining in numbers, are exposed to highly toxic trichlorfon. Exposure potentially occurred through different routes, most likely dermal and/or respiratory.

Non-dietary routes of pesticide exposure are poorly understood and represent a major component of bird exposure to insecticides [53]. Previous research has linked non-dietary exposure to pesticides with health risks in individual bird species. For instance, research in California suggests that red-tailed hawks (*Buteo jamaicensis*) in nut orchards were exposed to organophosphate insecticides, mainly through their feet [54], and that 75% of the hawks trapped in the study area had organophosphate metabolites in their faeces [55]. In a recent study, azinphos-methyl residues were detected in the skin, feathers and feet of brown-headed cowbirds (*Molothrus ater*) entering an apple orchard soon after application [56]. Driver et al. showed that for the insecticide methyl parathion, dermal uptake was the principal exposure route under normal crop spraying conditions [57]. In another study, zebra finches (*Poephila guttata*) exposed to ultralow volume applications of fenitrothion (with no dietary input) showed cholinesterase depressions similar to that found in wild birds exposed to forest sprays [58]. Thus, it is likely that a large number of bird species recorded foraging in macadamia orchards in this study, particularly at 24 h after spraying when residues were higher, were exposed to the insecticide through dermal and/or respiratory routes. These results are particularly relevant as trichlorfon has been found to have the highest dermal toxicity among a total of 35 insecticides, including highly toxic ones such as carbofuran or parathion [59]. Our results call for further research to clarify the consequences of non-dietary routes of insecticide exposure to avian communities at a global scale.

4.3. Impacts of Organophosphates on Arthropods

Trichlorfon induced changes in the arthropod communities inhabiting the orchards, but the apparency of the effects varied according to the trapping device used. Canopy arthropods captured with sweep-nets were impacted by the insecticide one day after the application, as indicated by significant reductions in total arthropod dry-weight, but not abundance (although the temporal trends were similar). Additionally, 'Time × Spray' interactions for canopy-dwellers (i.e., arthropods captured with sweep-nets), and ground-dwellers (i.e., arthropods captured with pit-fall traps) showed that trichlorfon produced a strong, but time-dependent impact on these two arthropod groups. Changes in the community structure of ground-dwelling arthropods were apparent three days post-application, whereas the canopy-dweller community was affected earlier by the insecticide (i.e., one day after the application), presumably as a consequence of the direct spray of insecticide over the macadamia foliage. These variable responses could have resulted from differences in susceptibility to trichlorfon, although 'sub-lethal' effects such as changes in arthropod performance and behaviour may also have occurred [60]. In contrast to canopy- and ground-dwellers, our results found no effect of trichlorfon on arthropods that were attracted to light-traps. The authors of a work studying the impact of trichlorfon on a number of selected invertebrate taxa in alfalfa suggested that differences in the insecticide-induced effect on arthropods could be related to differences in their mobility [31]. In this study, most of the captures in light-traps were flying arthropods which arguably possessed higher mobility than flightless, arboreal or epigeal taxa, thus supporting the hypothesis of the latter study that insects in light traps fly in from areas outside the application site. This hypothesis is also supported by the fact that the most affected arthropods in the present study were spiders.

Previous research has shown that the impact of broad-spectrum insecticides on non-target arthropods can ultimately disrupt the efficiency of ecosystem services, such as the crop pollination services provided by bees [61], or pest-regulation services provided by natural enemies [62]. Orchards support large numbers of spiders, possibly as a result of the complex vegetation structure of this crop (e.g., [63]); therefore opting for more selective insecticides can promote the long-term maintenance of spider numbers. In our study, trichlorfon applications significantly reduced the numbers of spiders, which are widely recognized predators of insect pests in a number of crops [64]. Other studies have found that broad-spectrum insecticides (other than trichlorfon) reduce the abundance and size of predatory spiders, concluding that pesticides, even when they are only applied early in the season, can have a significant long-term negative impact on spider abundance in orchards [65]. In our study, spiders were highly abundant in the canopies of macadamia trees, but their numbers were reduced by approximately 53% within 3 days of trichlorfon applications.

4.4. Relation between Arthropods and Bird Acoustic Activity

Approximately 80% of the bird species recorded during this study were either fully or partially insectivorous. Crisol-Martínez et al., showed that 11 bird species (all commonly recorded in most locations sampled in this study) all had arthropod-based diets, composed by several (40) families in eight arthropod orders [28]. Only one species, *Geopelia humeralis*, among the 19 most common species recorded in this study, does not generally include insects in its diet [66]. These results strongly suggest that the activity of bird communities in macadamia orchards is driven by food (arthropod) availability.

Overall, the present study suggests that after trichlorfon applications, birds continued to prey upon arthropods that had not yet died, or otherwise survived the immediate insecticide applications. These may have included arthropods that avoided the sprays (through microhabitat selection or because they arrived to the sites after spraying), or that were contaminated by the insecticide, but resistant to its effects. Our experimental design did not allow us to directly relate contaminated arthropods to probabilities of bird predation. A study in England showed that tree sparrows (*Passer montanus*) switched their diet to include significantly higher proportions of untreated Hemiptera (mainly aphids) following organophosphate insecticide applications [67]. Whereas "conditioned taste aversion" to insecticide-treated food resources may reduce the risk imposed to birds [68], there remains a possibility

that birds were feeding on insecticide-treated arthropods during this study. Several studies have recorded birds expiring or presenting secondary poisoning after consuming insects tainted with lethal amounts of insecticide (e.g., [69–71]). Although we cannot rule out this possibility of pesticide-induced bird mortality, we did not find any bird carcasses during the present study. Nonetheless, on four occasions we noted several individuals of *Gymnorhina tibicen* and *Corcorax melanorhamphos* foraging profusely on the ground a few hours after trichlorfon applications. Thus, sub-lethal effects on birds via ingestion may have also occurred. A single, low-dose (equivalent to that found in insecticide-treated insects) of trichlorfon can produce acute and chronic health impacts on avian populations, which could result in reductions in bird numbers [35], particularly during the breeding season. Therefore, reducing the use of broad-spectrum organophosphate insecticides, or switching to more wildlife-friendly pest management strategies, could help conserve the biodiversity in these and other agricultural systems.

5. Conclusions

Using the case of the macadamia industry in eastern Australia, the current study applied a novel bioacoustics methodology to show that a highly diverse community of bird species forages in orchards adjacent to forested habitats, and that their activity seems to be related to the arthropods inhabiting the orchards. Certain groups of arthropods were negatively impacted by trichlorfon, but the treatment did not alter avian activity. Two conclusions arise from these results, (i) in the short-term, birds are not indirectly affected by insecticide applications through reductions in food resources, probably because the most common bird species foraged on arthropods that were not affected by trichlorfon; but, (ii) because their activity is the same pre- and post-treatment, birds are directly exposed to the insecticide. The strong association between avian activity and arthropod communities suggests that ingestion of insecticide-tainted arthropods also occurred. Although the topic remains understudied, research has indicated that birds and other wildlife may be impacted by insecticides through a range of exposure routes other than ingestion. Our results raise several questions that require further investigation, particularly regarding the routes of exposure, the magnitude of exposure pathways, and the potential impact on birds at both the individual and the community levels. Orchard systems adjacent to forested habitats can be foraging grounds for a large diversity of birds (e.g., [46,47]). Our study supports previous findings (e.g., [52]), that stress the need to avoid organophosphates (and other broad-spectrum insecticides) in pest-management programmes in such biodiverse agricultural areas. By using wildlife-friendly pest-management options (e.g., cultural, physical and biological controls, as well as biopesticides) farmers could benefit from avian pest-reduction services (e.g., [28,72]) while supporting on-farm bird conservation (e.g., [73,74]). Thus, we recommend the adoption of insecticide-free management in orchards, particularly in areas adjacent to natural habitats to better protect birds and other wildlife from the adverse effects of pesticides.

Supplementary Materials: The following is available online at <http://www.mdpi.com/1/1/5/s1>, Table S1: Avian species recorded during the study, with the number of locations in which each species was found, and individual averaged abundance ratio across time (average \pm SE), based on 10-min point-counts.

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Appendix A

Trichlorfon is an organophosphate insecticide which is widely used in agriculture worldwide since the 50 s [3]. According to the Encyclopedia of Toxicology by Karanth (2014), trichlorfon is currently used on several field crops and foods, including also several developing countries. Trichlorfon is sold under various product names, including—but not limited to—Anthon[®], Bovinos[®], Briten[®], Chlorophos[®], Dyllox[®], Dyrex[®], Leivasom[®], Neguvon[®], Proxol[®], Totalene[®], and Trinex[®]. In Australia, during the period when the study was conducted, Lepidex[®] was a commercial insecticide product (500 g/L trichlorfon) commonly used in macadamia orchards—as well as in other crops [30]. Nowadays, Lepidex[®] use has been discontinued. However, Dipterex[®], containing exactly the same formulation as the previous product (trichlorfon 500 g/L), is commercialized and its use is recommended against “a wide variety of insect pests in various situations”, including three pests of macadamia, according to the producer information sheet (see https://cdn.nufarm.com/wp-content/uploads/sites/22/2018/05/14075246/Dipterex_500SL_label.pdf).

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