



## Pesticides drive differential leaf litter decomposition and mosquito colonisation dynamics in lentic conditions



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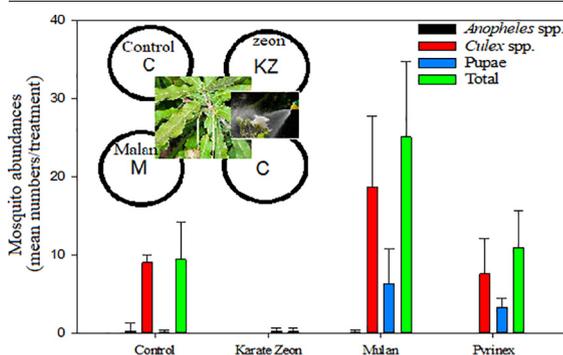
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### HIGHLIGHTS

- *Macadamia integrifolia* leaf litter decomposed faster when exposed to pesticides.
- Macadamia pesticides (Karate Zeon and Mulan) caused reductions in chlorophyll-*a* concentrations.
- Pyrinex and Mulan treatments promoted high mosquito (i.e., *Anopheles* spp., *Culex* spp.) abundances.
- Findings demonstrate macadamia pesticides may exert pressure on adjacent freshwater communities.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Global contamination of freshwater ecosystems by chemical compounds, such as pesticides, may exert high pressure on biologically-driven organic matter decomposition. These pollutants may also impair the quality of organic substrates for colonising invertebrates and reduce primary productivity by decreasing the abundance of phytoplankton. In southern Africa, increasing pesticide usage associated with macadamia plantations, in particular, presents a growing risk to freshwater ecosystems. Here, we examined macadamia (*Macadamia integrifolia*) leaf litter decomposition following exposure to three pesticides (i.e., Karate Zeon 10 CS (lambda-cyhalothrin), Mulan 20 AS (acetamiprid), Pyrinex 250 CS (chlorpyrifos)) used commonly in macadamia plantations, via an ex-situ microcosm approach. We examined mosquito colonisation of these microcosms as semi-aquatic macroinvertebrates which form a significant component of aquatic communities within standing waters. Macadamia leaf litter tended to decompose faster when exposed to Karate and Pyrinex pesticide treatments. Additionally, chlorophyll-*a*, conductivity, total dissolved solids, and pH differed among pesticide treatments and controls, with pesticides (Karate Zeon and Mulan) tending to reduce chlorophyll-*a* concentrations. Overall, pesticide treatments promoted mosquito (i.e., *Culex* spp.) and pupal abundances. In terms of dominant aquatic mosquito group abundances (i.e., *Anopheles* spp., *Culex* spp.), the effect of pesticides differed significantly among pesticide types, with Pyrinex and Mulan treatments having higher mosquito

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abundances in comparison to Karate Zeon and pesticide-free treatments. These findings collectively demonstrate that common pesticides used in the macadamia plantation may exert pressure on adjacent freshwater communities by shaping leaf-litter decomposition, semi-aquatic macroinvertebrate colonisation dynamics, and chlorophyll-*a*.

## 1. Introduction

Agriculture depends mostly on artificial lentic freshwater ecosystems as a source of water for irrigation purposes, such as impounded ponds, reservoirs and lakes (Dalu et al., 2012; Saulnier-Talbot and Lavoie, 2018; Cantonati et al., 2020). These freshwater ecosystems are also typically prone to threats associated with human activities (Olden et al., 2010). Freshwater pollution resulting from anthropogenic activities has become a widespread phenomenon over the past decades. Among pollution sources, agriculture intensification coupled with changes in practices to increase agricultural yields has led to massive inputs of toxicants into many aquatic ecosystems (Vörösmarty et al., 2010; Becker and Liess, 2017). Pesticide inputs may seriously threaten aquatic communities when they contaminate the sediment, water and vegetation. Pesticides can be harmful to various taxa, including fish, invertebrates, plants and even microbial communities, with implications for the entire food web (Rossi et al., 2018; Wasserman et al., 2016).

Agricultural pesticides are known to significantly impact the composition of aquatic communities and ecosystem processes (Rasmussen et al., 2012; Dalu and Tavengwa, 2022). The exposure of communities and ecosystem processes to agricultural pesticides has been shown to reduce primary production, with consequent impacts on invertebrates and other components of food webs (Rasmussen et al., 2012). Primary production and leaf litter decomposition are two key complementary ecosystem processes that ensure organic matter turnover, nutrient cycling and the provisioning of many ecosystem services. These two key processes also play a critical role in aquatic ecosystems (Hooper et al., 2012; Mutshekwa et al., 2020). Some freshwater aquatic ecosystems receive litter from the terrestrial ecosystems. In agricultural landscapes, much of this litter can come from crops which rely on pesticides spray for insect pest control. Experimental studies suggest that agricultural pesticides can alter microbial and detritivore decomposer communities with implications for litter decomposition rates (Zubrod et al., 2014; Cornejo et al., 2020). These processes likely affect aquatic food webs and habitats through reduced nutrient release dynamics and increased detrital accumulation (Shurin et al., 2006). As pesticide use increases, there is a pressing need to understand how aquatic ecosystem processes respond to these pollutants, particularly in the tropics where such studies are scant.

The macadamia, *Macadamia integrifolia* (Maiden and Betch), is an evergreen tree nut species native to Australia with important commercial value (Borompichaichartkul et al., 2009). South Africa is the largest macadamia producer globally (50,133 ha under cultivation and 50,000 t of production per year), followed by Australia and the USA, and with China and some South American and African countries growing fast in production (Macadamias South Africa (SAMAC), 2020). In South Africa the industry is predominantly spread over the Mpumalanga (44%), KwaZulu-Natal (28%) and Limpopo (20%) provinces (Macadamias South Africa (SAMAC), 2020). Rapid increases in macadamia plantations have resulted in the significant removal of natural vegetation to make space for macadamia plantations over the last decade (Schoeman, 2009; Linden et al., 2019; Linden, 2019). Given the rapid growth of a highly profitable macadamia industry in South Africa, it is essential to investigate associated horticultural practice impacts on nearby aquatic ecosystems.

To the best of our knowledge, no studies have examined the impacts of pesticides on *M. integrifolia* leaf litter decomposition and associated aquatic ecosystem communities. This study used an ex-situ microcosm approach to examine the effects of commonly-used pesticides (i.e., Karate Zeon 10 CS, Mulan 20 AS, Pyrinex 250 CS) in macadamia plantations on leaf decomposition under lentic conditions. We further assess macroinvertebrate establishment associated with the pesticide treatments, using a common,

ubiquitous and highly mobile semi-aquatic invertebrate taxon (i.e., mosquitoes) as a proxy for potential effects on colonisation. We further assessed primary production (i.e., chlorophyll-*a*) associated with pesticide treatments over time. We explored these pesticides individually to test our hypotheses that (i) *M. integrifolia* leaf litter decomposition will vary among pesticide treatments and that pesticide-free treatments will decompose fastest, due to adverse pesticide effects on decomposition and/or colonisation, (ii) phytoplankton biomass will differ among pesticide treatments with pesticide-free environments having the highest levels of chlorophyll-*a*, and (iii) exposure of leaf litter to pesticides reduces invertebrate colonisation.

## 2. Materials and methods

### 2.1. Pesticide background

The three pesticides used in the experiment were Karate Zeon 10 CS (Syngenta, Pretoria), Mulan 20 SP (ADAMA, Johannesburg), and Pyrinex 250 CS (ADAMA, Johannesburg). Karate Zeon has lambda-cyhalothrin (pyrethroid) as an active ingredient, whereas, Mulan has acetamiprid (neonicotinoid) and Pyrinex has chlorpyrifos (organophosphate). The three pesticides were purchased in local agricultural stores authorised for the sale and distribution of agrochemicals. Pyrinex and Karate Zeon are capsule suspension contact and stomach pesticides for the control of macadamia insects, such as thrips (*Scirtothrips aurantia*), cotton aphid (*Aphis gossypii*), and stink bugs (various species). Mulan is a water-soluble powder, systemic, contact and stomach insecticide to control pests on macadamia, canola, citrus, tomatoes, wheat, barley, oats, cotton and rooibos tea. The spray mixture (or dilution) volumes of 200 mL per 100 L water, 5 mL per 100 L water, and 40 g per 100 L water are for Karate Zeon 10 CS, Mulan 20 SP, and Pyrinex 10 CS, respectively, and according to the manufacturers' recommendations. These pesticides can be applied by any medium or high-volume applicator, aerially or on the ground. The applicator is correctly calibrated and fitted with an efficient agitation mechanism. These pesticides were chosen for this study because they are the most commonly-used pesticides by macadamia farmers in the Luvuvhu River Valley area for pest control (Nortjé et al., 2017). The pesticide concentrations in the current study were diluted in a 50 mL container filled with 30 mL of water and 0.5 mL hydrobuff (Nutrico, Johannesburg), which helps maintain the pH level to increase the efficacy of the solution, and 0.5 mL wetta (Nutrico, Johannesburg), thus promoting adherence to leaf litter.

### 2.2. Study area

The study was conducted from November to December 2020 at the Department of Geography and Environmental Sciences (−22.977580, 30.443819), the University of Venda, Limpopo Province of South Africa. Newly fallen *M. integrifolia* leaf litter with no sign of herbivory or decay were collected in late October 2020 from beneath trees by hand, given that many plants reabsorb nutrients from the leaves before senescence (Staaf and Berg, 1982) in the macadamia orchard (−23.058085, 30.280568), Tshakhuma village, Limpopo Province, South Africa. Only leaves with no sign of herbivory or decay were collected. After collection, leaf litter was air-dried at room temperature. Borehole water was collected from the Agriculture Department within the university campus, and river water was collected from the nearest water body (−22.982908, 30.442826). The area has a humid, subtropical climate and receives an average annual rainfall range of between 400 and 800 mm, with peak rainfall between 1000 and 1500 mm occurring in January and February. High

temperatures (i.e., up to 40 °C) occur between October and March, with the cool-dry season temperatures ranging between 12 °C and 22 °C. The area soil type is loam, which is red due to iron oxide.

### 2.3. Experimental design

The experiment was performed using a microcosm approach under controlled conditions. We used 40 × 10 L containers to represent four treatment types (i.e., ten replicates × 3 pesticides + controls without pesticides). After drying, approximately 3 g of the leaf material was weighed out using a RADWAG WPS 2100/C/1 precision balance, for inclusion in each bucket. Each leaf bundle (3 g) was then sprayed with 10 mL pesticide volume using foliar plant spray with different pesticides, following field concentrations that the macadamia farmers use i.e., Karate (100 µL), Mulan (500 mg) and Pyrinex (4500 µL) and recommendations by the macadamia farmers. Control leaf litter was sprayed with distilled water to stimulate the spraying treatment of pesticide leaf litter. The leaf litter was then left for 24 h to allow for chemicals to dry on the litter to avoid chemicals being washed off. Leaf litter bundles (3 g each) were then added into 10 L white polyethylene buckets (white plastic buckets; 20 cm diameter at the base, 24 cm high; leaf litter mass to water ratio 1:100), filled with 9 L of filtered (filter size 63 µm) borehole + river water (70:30 ratio). Leaves were made to sink by hand upon inoculation and buckets were topped up to the 9 L water level mark every week with filtered (GF/F filter 0.02 µm, Ø 47 mm) borehole water to replenish water lost due to evaporation. River water was characterised by temperature 27.3 ± 0.2 °C, conductivity 263.5 ± 4.0 µS cm<sup>-1</sup>, total dissolved solids 127 ± 2.6 mg L<sup>-1</sup> and pH 6.85 ± 0.1 and borehole water was characterised by temperature 27.2 ± 0.2 °C, conductivity 169.3 ± 4.0 µS cm<sup>-1</sup>, total dissolved solids 85.5 ± 2.6 mg L<sup>-1</sup> and pH 6.6 ± 0.1. The initial mean (n = 10) water temperature in the buckets was 25 ± 0.2 °C (SE), conductivity 232 ± 4.0 µS cm<sup>-1</sup>, total dissolved solids 115 ± 2.6 mg L<sup>-1</sup> and pH 8.3 ± 0.1. Once inoculated with treated leaf litter, all buckets were placed outside in an open rooftop area in a randomised array, at the Environmental Sciences building, University of Venda, approximately 0.5 km away from the nearest water body.

The experiment ran for 8 weeks. Water parameters (i.e., conductivity (µS cm<sup>-1</sup>), total dissolved solids (mg L<sup>-1</sup>), pH and temperature (°C) were measured on the first day of the experiment and every 7 days for the following 7 weeks using a multi-parameter handheld waterproof Cyber Scan 300 (Eutech Instruments, Cape Town, South Africa). In the eighth week, water was collected in each bucket using 100 mL container (n = 2) and then filtered (vacuum of < 5 cm Hg) through a 0.7 µm Whatman GF/F filter for chl-a determination (see below). After filtration, all mosquito larvae and pupae were collected, including via rinsing from the container. All mosquitoes were immediately transferred to 80% ethanol, for subsequent identification. Mosquito larvae were broadly identified to genus level using morphological features, mainly of the siphon and head, following recommendations by Jupp (1996). For abundance estimation among replicates, mosquitoes were enumerated according to taxa.

The remaining decomposed leaf litter was removed from the containers after 8 weeks using forceps. Each replicate per treatment was placed into a separate polyethylene zip (40 mm × 40 mm) to prevent loss of decomposed leaves. In the laboratory, decomposed leaf litter was then gently rinsed with sterilised distilled water to remove any algae and mosquito larvae, oven-dried at 60 °C for 48 h and weighed using RADWAG WPS 2100/C/1 precision balance, following recommendations by Harmon et al. (1999). Results were expressed as the percentage of initial oven-dry mass remaining. Additionally, approximately 3 g air-dried leaf litter samples (n = 10), which were not incubated in buckets and not sprayed, were oven-dried at 60 °C for 48 h to a constant mass to allow calculation of initial air-dry mass to initial oven-dry mass conversion factors, needed to estimate initial leaf litter oven-dry mass.

### 2.4. Water samples and chlorophyll-a concentration determination

After filtration (see above), the filters from water sampled for chl-a determination were inserted in separate labelled bottles containing 10 mL acetone and stored in the center of the atrium in an open space for 24 h to allow chl-a extraction (Hansson et al., 1998). Chlorophyll-a was measured as an estimate of phytoplankton biomass in duplicate polyethylene bottles (100 mL) filled with water from each leaf container. After 24 h, chl-a concentration was determined using SPECTROstar NANO (BMG LabTech GmbH, Ortenberg) according to Lorenzen (1967):

$$Chla \text{ (mg m}^{-3}\text{)} = 11.4 \times K \times ((665_o - 750_o) - (665_a - 750_a)) \times V_e \div L \times V_f$$

where,  $L$  – cuvette light-path (cm),  $V_e$  – extraction volume (mL),  $V_f$  – filtered volume (L) and  $K$  – 2.43.

### 2.5. Statistical analysis

The effects of pesticide *treatment* (4 levels, including controls) and observation *week* (8 levels) on key water parameters (pH, conductivity, total dissolved solids and temperature) were examined using linear mixed-effects models. Individual containers were included as a random effect (intercept) to account for repeated measures over the experimental period. A Kruskal-Wallis test was used to compare the variation in mosquito larval and pupal groups among treatments (4 levels), as the Shapiro-Wilk test indicated non-normality. Remaining mass (final oven-dry mass: initial oven-dry mass) expressed as percentage of the leaf litter in each treatment was log-transformed prior to the analyses. Remaining mass (%) and chl-a concentrations were analysed separately using a one-way analysis of variance according to treatment (four levels). Tukey post-hoc tests were used to evaluate multiple comparisons among chl-a concentrations and remaining mass (%) where effects were significant. In all analyses, significance was inferred at  $p < 0.05$ . All statistical analyses were performed using IBM SPSS Statistics 28.0.0.0.

## 3. Results

### 3.1. Environmental variables

Across treatments, conductivity (204.1–551.8 µS cm<sup>-1</sup>), total dissolved solids (101.9–243.1 mg L<sup>-1</sup>), pH (5.9–8.9) and temperature (range 25–35.4 °C) differed substantially (Table 1). Conductivity, total dissolved solids and pH, measured throughout the monitoring period, differed between treatments and over time, but with no treatment × time interactions (Tables 1, 2). Control and pesticide treatments i.e., Karate Zeon, Mulan and Pyrinex were all significantly different pairwise ( $p < 0.05$ ) in terms of pH, conductivity and total dissolved oxygen (Table 1). Temperature, however, differed only according to time and not treatment, indicating that treatment effects were not confounded by temperature variations.

### 3.2. Mosquito abundances

Overall, a total of 456 individual mosquitoes were counted across all treatments. *Culex* spp. comprised 77.4% of all mosquito numbers, with 0.9% identified as *Anopheles* spp. and 21.7% unidentified as they were at the pupal stage. No significant differences were detected for *Anopheles* spp. abundances across treatments (Kruskal-Wallis:  $H = 2.054$ ,  $df = 3$ ,  $p = 0.216$ ), whereas *Culex* spp. differed significantly (Kruskal-Wallis:  $H = 29.126$ ,  $df = 3$ ,  $p = 0.004$ ). Larval mosquito numbers were by far the highest in Mulan and followed by Pyrinex, control and then Karate Zeon (Fig. 1). Mosquito pupae of both genera differed significantly across treatments (Kruskal-Wallis:  $H = 31.848$ ,  $df = 3$ ,  $p = 0.018$ ). Mosquito pupae numbers were by far the highest in Mulan and followed by Pyrinex, Karate Zeon and then control (Fig. 1).

**Table 1**  
Mean ( $\pm$  standard deviation) ( $n = 10$ ) of environmental parameters measured across treatments over time (8 weeks).

Parameters	Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Conductivity ( $\mu\text{S cm}^{-1}$ )	Control	323.0 $\pm$ 0	235.5 $\pm$ 1	252.9 $\pm$ 1.2	315.5 $\pm$ 2.4	546.4 $\pm$ 1.2	327.9 $\pm$ 1.4	278.5 $\pm$ 0.1	204.7 $\pm$ 0.7
	Karate Zeon	232.0 $\pm$ 0	225.0 $\pm$ 1.9	258.0 $\pm$ 4	319.9 $\pm$ 5.7	551.8 $\pm$ 6.2	340.8 $\pm$ 5.0	277.6 $\pm$ 2.8	205.8 $\pm$ 3.3
	Mulan	232.0 $\pm$ 0	227.6 $\pm$ 1.3	254.5 $\pm$ 1.4	307.1 $\pm$ 1.2	544.5 $\pm$ 2.1	326.0 $\pm$ 1.6	272.1 $\pm$ 1.2	204.1 $\pm$ 0.1
	Pyrinex	232.0 $\pm$ 0	236.9 $\pm$ 5.5	258.0 $\pm$ 6.3	318.7 $\pm$ 5.4	538.6 $\pm$ 16.8	275.7 $\pm$ 4.9	275.7 $\pm$ 4.9	206.3 $\pm$ 3.7
Total dissolved solids $\text{mg L}^{-1}$ )	Control	115.0 $\pm$ 0	115.7 $\pm$ 0.3	126.1 $\pm$ 0.6	155.1 $\pm$ 0.6	240.6 $\pm$ 0.9	163.0 $\pm$ 0.7	138.8 $\pm$ 0.4	102.8 $\pm$ 0.3
	Karate Zeon	115.0 $\pm$ 0	111.7 $\pm$ 1.1	128.7 $\pm$ 1.9	158.4 $\pm$ 2.3	243.1 $\pm$ 5.2	166.7 $\pm$ 2.9	138.4 $\pm$ 1.5	102.8 $\pm$ 1.7
	Mulan	115.0 $\pm$ 0	114.3 $\pm$ 0.6	126.9 $\pm$ 0.7	153.6 $\pm$ 0.6	233.9 $\pm$ 1.1	162.9 $\pm$ 0.8	124.9 $\pm$ 0.5	101.9 $\pm$ 0.5
	Pyrinex	115.0 $\pm$ 0	118.3 $\pm$ 3	128.7 $\pm$ 3.1	159.2 $\pm$ 2.6	240 $\pm$ 3.5	163.2 $\pm$ 1.9	113.8 $\pm$ 1.9	102.7 $\pm$ 1.9
pH	Control	8.2.0 $\pm$ 0	7.7 $\pm$ 0	7.7 $\pm$ 0	7.7 $\pm$ 0	8.8 $\pm$ 0	8.3 $\pm$ 0.4	6.5 $\pm$ 0	6.0 $\pm$ 0
	Karate Zeon	8.3.0 $\pm$ 0.1	8.0.0 $\pm$ 0	7.7 $\pm$ 0	7.7 $\pm$ 0	8.9 $\pm$ 0	7.00	6.8 $\pm$ 0.1	6.00.1
	Mulan	8.3.0 $\pm$ 0	8.0 $\pm$ 0	7.8 $\pm$ 0	7.8 $\pm$ 0	8.9 $\pm$ 0	7.0 $\pm$ 0	6.7 $\pm$ 0	5.9 $\pm$ 0
	Pyrinex	8.3.0 $\pm$ 0.1	8.0 $\pm$ 0	7.8 $\pm$ 0	7.7 $\pm$ 0.1	8.9 $\pm$ 0.1	7.1 $\pm$ 0.1	6.6 $\pm$ 0.1	6.0 $\pm$ 0.1
Temperature $^{\circ}\text{C}$	Control	25.0 $\pm$ 0	33.1 $\pm$ 0	22.3 $\pm$ 0	30.2 $\pm$ 0.2	21.7 $\pm$ 0	19.8 $\pm$ 0	32.6 $\pm$ 0	35.0 $\pm$ 0
	Karate Zeon	25.0 $\pm$ 0	33.5 $\pm$ 0.5	22.2 $\pm$ 0	30.5 $\pm$ 0.2	22.0 $\pm$ 0.1	19.8 $\pm$ 0	32.9 $\pm$ 0.1	35.4 $\pm$ 0.2
	Mulan	25. $\pm$ 0	33.1 $\pm$ 0	22.2 $\pm$ 0	30.4 $\pm$ 0.3	21.8 $\pm$ 0	19.7 $\pm$ 0	32.9 $\pm$ 0.1	35.3 $\pm$ 0.1
	Pyrinex	25.0 $\pm$ 0	33.2 $\pm$ 0.1	22.3 $\pm$ 0	30.3 $\pm$ 0.1	21.7 $\pm$ 0.1	19.7 $\pm$ 0.1	32.7 $\pm$ 0.1	35.2 $\pm$ 0.2

**Table 2**  
Linear mixed-effects model results considering key water parameters as a function of leaf Treatment and Time (week), and their interaction. F-values are discerned with Type III sums of squares via Satterthwaite's method.

Parameter	Predictor	F-value	p-Value
Temperature	Treatment	1.23	0.310
	Time	2565.10	<0.001
	Treatment $\times$ Time	1.74	0.202
pH	Treatment	30.62	<0.001
	Time	4148.40	<0.001
	Treatment $\times$ Time	1.17	0.292
Conductivity	Treatment	1042.81	<0.001
	Time	12,988.78	<0.001
	Treatment $\times$ Time	0.23	0.637
Total dissolved solids	Treatment	185.80	<0.001
	Time	3497.23	<0.001
	Treatment $\times$ Time	1.40	0.251

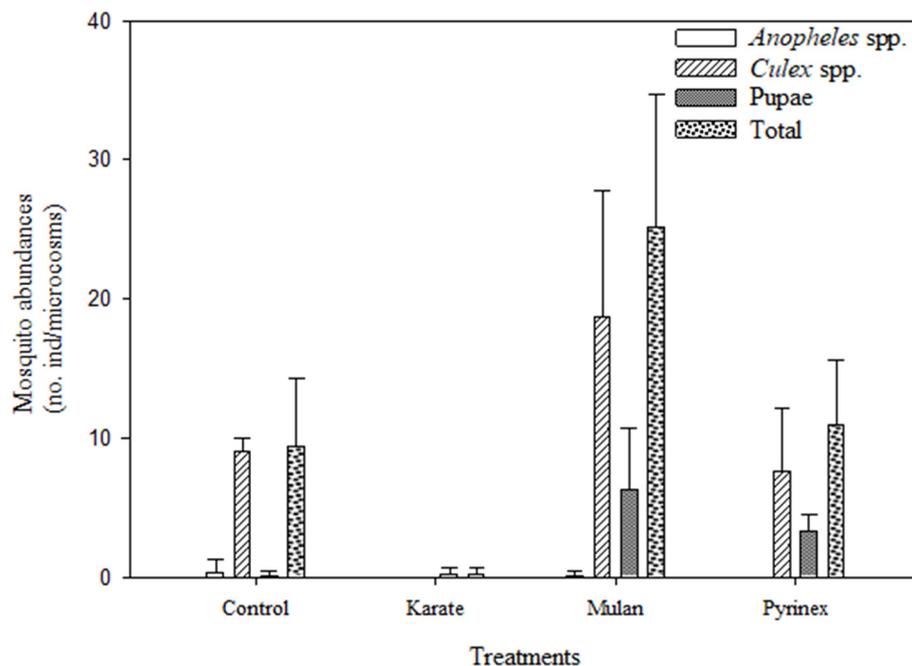
Significant p-values are in bold.

### 3.3. Chlorophyll-a concentration

Chlorophyll-a concentrations generally increased in control treatments compared to pesticide treatments (ANOVA:  $F = 0.472$ ,  $df = 3$ ,  $p = 0.033$ ) (Fig. 2). Pesticide-free control treatments had significantly higher mean chl-a concentration ( $0.30 \text{ mg L}^{-1}$ ) compared to Karate Zeon ( $0.02 \text{ mg L}^{-1}$ ) and Mulan ( $0.04 \text{ mg L}^{-1}$ ). Chlorophyll-a concentrations demonstrated differences among pesticide treatments following a decreasing order of Pyrinex > Mulan > Karate. Following Tukey's post-hoc analysis, significant differences were highlighted between treatments i.e., control vs Karate ( $p < 0.001$ ), control vs Mulan ( $p < 0.001$ ), Karate vs Pyrinex ( $p < 0.001$ ) and Mulan vs Pyrinex ( $p < 0.001$ ).

### 3.4. Decomposition

Leaf litter mass remaining (%) of *M. integrifolia* at the end of the experiment differed significantly among treatments i.e., control, Karate, Mulan, Pyrinex (ANOVA:  $F = 11.034$ ,  $df = 3$ ,  $p < 0.001$ ), following a decreasing



**Fig. 1.** Total (larvae + pupae), larval (*Culex* spp. and *Anopheles* spp.) and pupal (all genera) mosquito abundances (mean  $\pm$  SD,  $n = 10$ ) in 9 L containers across experimental treatments of *Macadamia integrifolia* leaf litter treated with Karate Zeon, Mulan, Pyrinex and pesticide-free controls at the end of the experiment (8 weeks). Abbreviation: ind – individual.

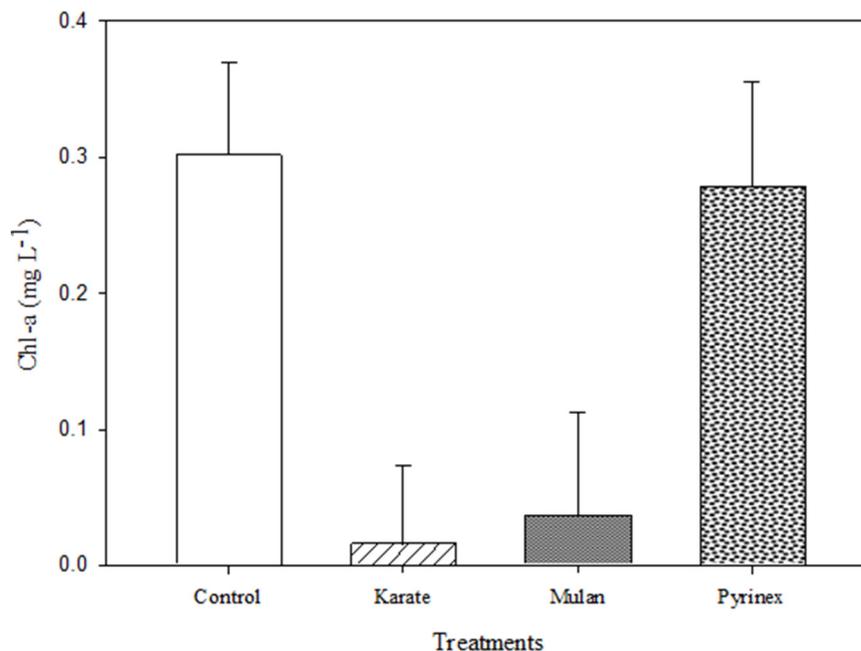


Fig. 2. Chlorophyll-a concentrations (mean  $\pm$  SD,  $n = 10$ ) from Karate Zeon, Mulan, Pyrinex, and control treatments at the end of the experiment (8 weeks).

order of Mulan (56.7%) < Control (77.8%) < Karate (84.8%) < Pyrinex (88.5%). Leaf litter treated with Mulan drove increased decomposition, with significantly reduced mass compared to Karate Zeon, Pyrinex and controls (all  $p < 0.001$ ) (Fig. 3). Following Tukey's post-hoc analysis, significant differences were highlighted between treatments i.e., control vs Mulan ( $p < 0.004$ ), Karate vs Mulan ( $p < 0.001$ ), Mulan vs Pyrinex ( $p < 0.001$ ).

#### 4. Discussion

Several studies have examined the impact of agricultural pesticides on streams, colonisation and aquatic ecosystem processes, providing vital insight into how they affect functioning and integrity (Artigas et al., 2012; Muturi et al., 2017; Cornejo et al., 2020; Cornejo et al., 2021). However, there had been research gaps on how different pesticides affect

aquatic ecosystem functioning and integrity in lentic systems. With increased plantation of macadamia near aquatic systems, understanding the effects of agricultural pesticides on macadamia leaf litter decomposition, primary production and colonisation is of high interest, to predict the consequences of changes on aquatic ecosystem functioning. Our results reveal diverse effects of pesticides commonly used in macadamia farms on macadamia leaf litter decomposition, associated primary productivity and invertebrate colonisation dynamics. Agricultural pesticides are known to contaminate streams and, together with associated stressors such as organic pollution and habitat loss, alter their assemblages (Fugère et al., 2016; Rasmussen et al., 2012; Cornejo et al., 2019) and impair their functioning (Dawoud et al., 2017; Cornejo et al., 2020; Fugère et al., 2020). Using an ex-situ microcosm experiment, overall, we found that Karate Zeon and Mulan had a significantly negative effect on water column primary

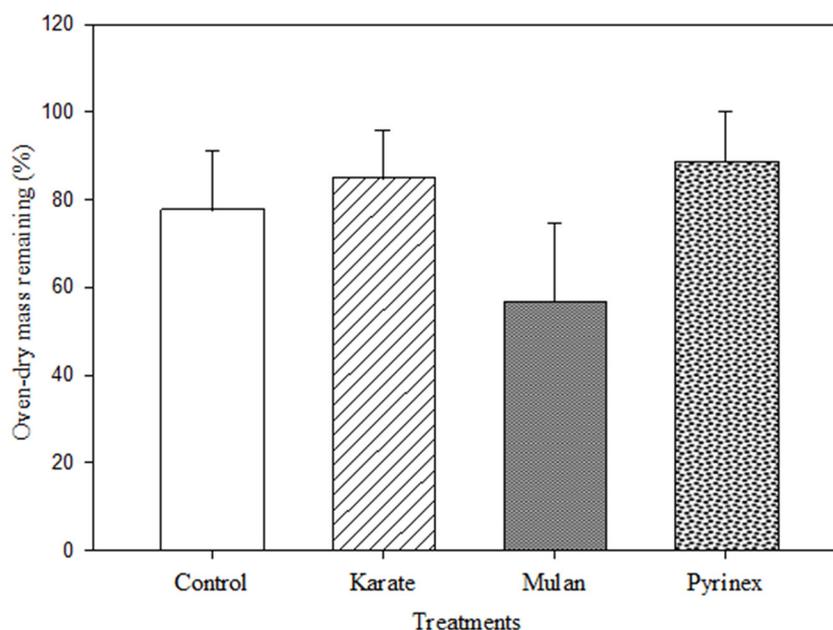


Fig. 3. Oven-dry mass remaining (mean  $\pm$  SD,  $n = 10$ ) of macadamia leaf litter treated with the pesticides Karate Zeon, Mulan, Pyrinex, and pesticide-free (controls) at the end of the experiment (8 weeks).

productivity (using chl-*a* concentrations as a proxy), while Pynrex seemed to have little effect when compared to control treatments and also effected conductivity, total dissolved solids, and pH significantly across treatments and weeks. Furthermore, we found that pesticide type strongly influenced *M. integrifolia* decomposition rates. Pesticide effects on decomposition were significantly higher for Mulan compared to Karate Zeon and Pynrex. Furthermore, Mulan and control treatments also differed significantly. Lastly, we found that select pesticides used in *M. integrifolia* plantation may promote the colonisation, development and/or growth of invertebrates, specifically mosquitoes. Consistent with previous work using microcosm approaches to mimic aquatic systems, pesticides generally affected decomposition, primary production and colonisation (e.g., Kominoski et al., 2007).

Environmental parameters can regulate invertebrate development and act as indicators of water quality (Morrissey et al., 2015). In this study, conductivity, total dissolved solids, and pH differed significantly across the experimental period and treatments. These measures show that the water-quality parameters we measured may have played a role in differential colonisation across treatments, primary production and decomposition. With regard to primary production, high levels of chlorophyll-*a* often indicate poor water quality and low levels often suggest good conditions; poor (chl-*a* > 20 µg L<sup>-1</sup>), fair (5 < chl-*a* < 20 µg L<sup>-1</sup>), good (chl-*a* < 5 µg L<sup>-1</sup>) (Huang et al., 2011). Consistently high or variable chlorophyll-*a* concentration may indicate the occurrence of algal blooms, which can be harmful to other aquatic organisms. During the present study, phytoplankton biomass varied among the different pesticide treatments and controls. In particular, Karate Zeon and Mulan caused significantly reduced chlorophyll-*a* concentrations whereas there was a high chlorophyll-*a* concentration in control and Pynrex treatments. The abundance of algal assemblages, which are important food resources for filter feeders such as mosquito larvae (Merritt et al., 1992), are affected by multiple interactive factors such as water chemistry (Stevenson et al., 1996).

Allochthonous inputs are crucial factors affecting resource availability within aquatic systems (Cuthbert et al., 2022). As expected, following a similar study by Muturi et al. (2013), exposure to pesticides affected decomposition significantly. In the present study, leaf litter decomposition, a key ecosystem process in freshwater environments, was reduced (11.5–43.3%) under pesticide treatments. While there is an absence of literature on the effects of agricultural pesticides on *M. integrifolia* leaf litter, several studies evaluating the effects of agricultural pesticides on various plant species have indicated that pesticides significantly reduced the rate of leaf litter decomposition (Hagen et al., 2006; Piscart et al., 2009; Magbanua et al., 2010; Muturi et al., 2013). A recent study by Sumudumali et al. (2022) reported a reduced mass loss of *Panicum maximum* leaf litter when exposed to agricultural pesticides. It has been well noted that the decomposition of litter is highly influenced by litter quality (in terms of Carbon: Nitrogen ratios), but also by climate (mainly temperature), and the composition of decomposer organisms. Leaf litter characteristics are of great importance in controlling both the short and long-term decomposition rates. Furthermore, microbial decomposition activity has been indicated to be affected by the availability of oxygen, with low oxygen levels often resulting in slow decomposition rates (Medeiros et al., 2009). The differences in the period of decomposition vary tremendously among plant species, with leaves that decompose slowly often having high leaf toughness scores (Ramos et al., 2021). However, we did not measure leaf toughness, C:N ratios or oxygen concentrations in this study. The direct cause of mass loss in the present study was unclear and we are unsure of different contributions (i.e., leaching, microbial or invertebrates), and that pesticides effects likely resulted from impaired biological activity. However, a predominance of fungi in microbial decomposers has been found in microcosms experiments (Hieber and Gessner, 2002; Gulis and Suberkropp, 2003; Afolabi et al., 2019). The fact that Mulan had increased decomposition indicates that the effects were due to the main active pesticide ingredient, i.e., acetamiprid (neonicotinoid). Variation in mass loss among pesticide leaf treatments

is not surprising, given that impact of pesticides on leaf litter decay can be influenced by pesticide toxicity to microorganisms (Rasmussen et al., 2012). Furthermore, increasing pesticide activity that is associated with agricultural production has been shown to decrease microbial diversity, and decomposition (DeLorenzo et al., 2001; Afolabi et al., 2019).

However, due to the nature of our experimental design, we could not determine whether the effect of pesticides on decay rates was driven by microbial communities, if any, present in the treatments since microbial communities were not assessed. As such, the mechanisms by which leaf litter degradation is affected are yet to be determined. Nonetheless, microbes have been recognised as playing a dominant role in leaf litter decomposition and acting as source of food for aquatic invertebrates (Merritt et al., 1992; Linenberg et al., 2016). It cannot be ruled out that microbes altered colonisation of mosquitoes and decomposition in pesticides treatment since they play a vital role in breaking down debris and act as a source of food for mosquitoes (Muturi et al., 2012).

Semi-aquatic invertebrates are an essential component of aquatic ecosystems. These taxa are found in or on a multitude of microhabitats in streams. A large portion of invertebrates in these systems comprises insects that externally colonise the terrestrial landscape, such as mosquitoes. The presence of mosquitoes in the treatments is not surprising given that several taxa (e.g., *Culex pipiens*) are referred to as container breeders, since they lay eggs in confined, artificial environments (Cuthbert et al., 2019). We demonstrated that *M. integrifolia* leaf litter inputs treated with pesticides, namely Karate Zeon, Mulan, Pynrex and controls, differed markedly in their effects on mosquito colonisation. These pesticides might adversely affect mosquito natural enemies, and therefore promote their proliferation through enemy release in aquatic environments (Govindarajan et al., 2018). We found that Mulan and Pynrex were by far the most favourable pesticides for the development of mosquitoes and caused elevated abundances of larvae and pupae. Elevated mosquito abundances in Mulan and Pynrex could be due to their efficacy being lower in water, as a result of their active ingredients i.e., acetamiprid (neonicotinoid) and chlorpyrifos (organophosphate), respectively, being deactivated (Huston and Pignatello, 1999). Although not tested here, these pesticides might also adversely affect mosquito natural enemies, and therefore promote their proliferation through enemy release.

The almost complete absence of *Anopheles* spp. and *Culex* spp. in Karate Zeon treatments could be due to the fact that pyrethroid insecticides cause rapid declines non-target organisms, and are known to hold dangerous toxic effects on the exposed organisms (Farag et al., 2021). However, low abundances of *Anopheles* spp. across all treatments could largely be due to their habitat preference for larger systems, i.e., not container-based habitats (Minakawa et al., 2004) or due to their egg differences since *Culex* spp. lay eggs one at a time in a raft of 100 to 300 eggs resulting in less capacity to exploit multiple habitats by an individual, whereas *Anopheles* spp. lay eggs singly at a time (Mbare et al., 2014). Generally, pyrethroids (in this case, Karate Zeon) are used against adult mosquitoes and are not suitable for controlling mosquito larvae due to their high fish toxicities. The reason for reduced colonisation could relate to habitat selection due to inadvertent contamination of the larval habitat with permethrin, but we did not test to what extent it was drive by colonisation differences or direct larval mortality post-arrival. Overall, high mosquito abundances and colonisation in Mulan treatments indicate better-perceived habitat quality for mosquitoes, such as through the more rapid decomposition and availability of organic matter as resources in the water we observed.

Like other ecological studies, this study had several limitations. First, we quantified the rate of decomposition and invertebrates abundances associated with *M. integrifolia* leaf litter exposed to pesticide concentrations applied directly to *M. integrifolia* plantations, not concentrations in the water bodies. Nonetheless, invertebrates are known to develop in reservoirs associated with high levels of anthropogenic disturbance (Jatulewicz, 2007; Firmiano et al., 2021). Future studies should examine the impact of *M. integrifolia* anthropogenic disturbances on invertebrates colonisation and microbial abundances exposed at environmentally-relevant concentrations. However, we believe our results are a good starting point to the

understanding of effects of such pesticides on aquatic habitats, invertebrate communities and abundances, primary production as well as decomposition dynamics.

## 5. Conclusions

In conclusion, we assessed variation in decomposition rates, mosquito colonisation and primary production levels i.e., chlorophyll-*a* among pesticide treatments, namely Karate Zeon, Mulan, and Pyrinex as indicators for habitat quality. We found that pesticide treatments induced shifts in decomposition, colonisation and primary production, but these effects varied among pesticide type. The variety of litter decomposition across treatments would be most likely due to leaching. However, leaching levels were not measured. Although not assessed, we can further conjecture that mass loss could have been influenced by the capacity of microbial decomposers to endure pesticides and, thus influence the nutrient levels available to mosquito larvae. These findings provide a basis for understanding the implications of pesticides in *M. integrifolia* plantations and aquatic ecosystem processes. The findings are also essential for health sectors in understanding how anthropogenic chemical contaminants may affect human health since container aquatic habitats are mostly utilised by humans and are dominated by mosquitoes, which transmit a wide variety of life-threatening human pathogens and parasites (Cox-Singh et al., 2008). Based on our results, continuously increased plantation of *M. integrifolia* trees and particular usage of Karate Zeon as pest control will likely have a considerable effect on invertebrate colonisation. However, interactions between leaf type, pesticides and invertebrates can be highly complex and difficult to predict. We, therefore, propose that it is essential to consider risks to invertebrate communities in aquatic ecosystems during the production of pesticides. The use of pesticides in agriculture represents an important selective force likely to affect all types of aquatic organisms. Therefore, further studies are required to directly link mosquito abundance and community structure associated with detritus types and pesticide exposure. Furthermore, the current findings stress the need for studies on macadamia leaf litter chemical characteristics to help give insight of decomposition dynamics of macadamia leaf litter and the effects on functioning of freshwater ecosystems and associated services.

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## Data availability statement

The datasets generated and/or analysed during the current study are not publicly available as they are part of larger study that is currently ongoing but are available from the corresponding author on reasonable request.

## CRediT authorship contribution statement

**Thendo Mutshekwa:** Formal analysis, Methodology, Data curation, Writing – original draft, Writing – review & editing. **Lutendo Mugwedi:** Methodology, Visualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Ryan J. Wasserman:** Visualization, Methodology, Supervision, Writing – review & editing. **Ross N. Cuthbert:** Visualization, Methodology, Writing – review & editing. **Farai Dondofema:** Methodology, Visualization, Investigation, Supervision, Writing – review & editing. **Tatenda Dalu:** Conceptualization,

Investigation, Methodology, Data curation, Formal analysis, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

- Afolabi, O.K., Aderibigbe, F.A., Folarin, D.T., Arinola, A., Wusu, A.D., 2019. Oxidative stress and inflammation following sub-lethal oral exposure of cypermethrin in rats: mitigating potential of epicatechin. *Heliyon* 5, 02274.
- Artigas, J., Majerholc, J., Foulquier, A., Margoum, C., Volat, B., Neyra, M., Pesce, S., 2012. Effects of the fungicide tebuconazole on microbial capacities for litter breakdown in streams. *Aquat. Toxicol.* 122, 197–205.
- Becker, J.M., Liess, M., 2017. Species diversity hinders adaptation to toxicants. *Environ. Sci. Technol.* 51, 10195–10202.
- Borompichaichartkul, C., Luengsoke, K., Chinprahast, N., Devahastin, S., 2009. Improving quality of macadamia nut (*Macadamia integrifolia*) through the use of hybrid drying process. *J. Food Eng.* 93, 348–353.
- Cantonati, M., Poikane, S., Pringle, C.M., Stevens, L.E., Turak, E., Heino, J., Richardson, J.S., Bolpagni, R., Borrini, A., Cid, N., Čtvrtíková, M., 2020. Characteristics, main impacts, and stewardship of natural and artificial freshwater environments: consequences for biodiversity conservation. *Water* 12, 260.
- Cornejo, A., Tonin, A.M., Checa, B., Tuñón, A.R., Pérez, D., Coronado, E., González, S., Ríos, T., Macchi, P., Correa-Araneda, F., Boyero, L., 2019. Effects of multiple stressors associated with agriculture on stream macroinvertebrate communities in a tropical catchment. *PLoS One* 14, 0220528.
- Cornejo, A., Pérez, J., Alonso, A., López-Rojo, N., Monroy, S., Boyero, L., 2020. A common fungicide impairs stream ecosystem functioning through effects on aquatic hyphomycetes and detritivorous caddisflies. *J. Environ. Manag.* 263, 110425.
- Cornejo, A., Pérez, J., López-Rojo, N., García, G., Pérez, E., Guerra, A., Nieto, C., Boyero, L., 2021. Litter decomposition can be reduced by pesticide effects on detritivores and decomposers: implications for tropical stream functioning. *Environ. Pollut.* 285, 117243.
- Cox-Singh, J., Davis, T.M., Lee, K.S., Shamsul, S.S., Matusop, A., Ratnam, S., Rahman, H.A., Conway, D.J., Singh, B., 2008. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clin. Infect. Dis.* 46, 165–171.
- Cuthbert, R.N., Dalu, T., Mutshekwa, T., Wasserman, R.J., 2019. Leaf inputs from invasive and native plants drive differential mosquito abundances. *Sci. Total Environ.* 689, 652–654.
- Cuthbert, R.N., Wasserman, R.J., Keates, C., Dalu, T., 2022. Food webs. In: Dalu, T., Wasserman, R.J. (Eds.), *Fundamentals of Tropical Freshwater Wetlands: From Ecology to Conservation Management*. Elsevier, Cambridge.
- Dalu, T., Tavengwa, N.T. (Eds.), 2022. *Emerging Freshwater Pollutants: Analysis, Fate and Regulations*. Elsevier, Cambridge.
- Dalu, T., Clegg, B., Nhiwatiwa, T., 2012. Macroinvertebrate communities associated with littoral zone habitats and the influence of environmental factors in Malilangwe Reservoir, Zimbabwe. *Knowl. Manag. Aquat. Ecosyst.* 406, 06.
- Dawoud, M., Bundschuh, M., Goedkoop, W., McKie, B.G., 2017. Interactive effects of an insecticide and a fungicide on different organism groups and ecosystem functioning in a stream detrital food web. *Aquat. Toxicol.* 186, 215–221.
- DeLorenzo, Marie, E., Geoffrey, I., Scott, P., Ross, E., 2001. Toxicity of pesticides to aquatic microorganisms: a review. *Environ. Toxicol. and Chem.* 20, 84–98.
- Farag, M.R., Alagawany, M., Bilal, R.M., Gewida, A.G., Dhama, K., Abdel-Latif, H.M., Amer, M.S., Rivero-Perez, N., Zaragoza-Bastida, A., Binnaser, Y.S., Batiha, G.E.S., 2021. An overview on the potential hazards of pyrethroid insecticides in fish, with special emphasis on cypermethrin toxicity. *Animals* 11, 880.
- Firmiano, K.R., Castro, D.M., Linares, M.S., Callisto, M., 2021. Functional responses of aquatic invertebrates to anthropogenic stressors in riparian zones of neotropical savanna streams. *Sci. Total Environ.* 753, 141865.
- Fugère, V., Kasangaki, A., Chapman, L.J., 2016. Land use changes in an afro-tropical biodiversity hotspot affect stream alpha and beta diversity. *Ecosphere* 7, e01355.
- Fugère, V., Lostchuck, E., Chapman, L.J., 2020. Litter decomposition in afro-tropical streams: effects of land use, home-field advantage, and terrestrial herbivory. *Freshw. Sci.* 39, 497–507.
- Govindarajan, M., Rajeswary, M., Senthilmurugan, S., Vijayan, P., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., Benelli, G., 2018. Larvicidal activity of the essential oil from *Amomum subulatum* roxb. (Zingiberaceae) against *Anopheles subpictus*, *Aedes albopictus* and *Culex tritaeniorhynchus* (Diptera: Culicidae), and non-target impact on four mosquito natural enemies. *Physiol. Mol. Plant Pathol.* 101, 219–224.
- Gulis, V., Suberkropp, K., 2003. Interactions between stream fungi and bacteria associated with decomposing leaf litter at different levels of nutrient availability. *Aquat. Microb. Ecol.* 30, 149–157.

- Hagen, E.M., Webster, J.R., Benfield, E.F., 2006. Are leaf breakdown rates a useful measure of stream integrity along an agricultural landuse gradient? *J. N. Am. Benthol. Soc.* 25, 330343.
- Hansson, L.A., Brönmark, C., Nyström, P., Greenberg, L., Lundberg, P., Nilsson, P.A., Persson, A., Pettersson, L.B., Romare, P., Tranvik, L.J., 1998. Consumption patterns, complexity and enrichment in aquatic food chains. *Proc. R. Soc. London, Ser. B* 265, 901–906.
- Harmon, M.E., Nadelhoffer, K.J., Blair, J.M., 1999. Measuring decomposition, nutrient turnover, and stores in plant litter. In: Robertson, G.P., Bledsoe, C.S., Coleman, D.C., Sollins, P. (Eds.), *Standard Soil Methods for Long-term Ecological Research*. Oxford University Press, New York.
- Hieber, M., Gessner, M.O., 2002. Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology* 83, 1026–1038.
- Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L., O'Connor, M.I., 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature* 486, 105–108.
- Huang, W., Mukherjee, D., Chen, S., 2011. Assessment of hurricane Ivan impact on chlorophyll-a in Pensacola Bay by MODIS 250 m remote sensing. *Mar. Pollut. Bull.* 62, 490–498.
- Huston, P.L., Pignatello, J.J., 1999. Degradation of selected pesticide active ingredients and commercial formulations in water by the photo-assisted Fenton reaction. *Water Res.* 33, 238–246.
- Jatulewicz, I., 2007. Comparison of macroinvertebrate communities associated with various habitats in anthropogenic reservoirs. *Oceanol. Hydrobiol. Stud.* 36, 39–47.
- Jupp, P.G., 1996. Mosquitoes of Southern Africa: Culicinae and Toxorhynchitinae. *Ekogilde Publishers, Hartebeespoort*, p. 156.
- Kominoski, J.S., Pringle, C.M., Ball, B.A., Bradford, M.A., Coleman, D.C., Hall, D.B., Hunter, M.D., 2007. Non-additive effects of leaf litter species diversity on breakdown dynamics in a detritus-based stream. *Ecology* 88, 1167–1176.
- Linden, V.M., 2019. How vertebrate communities affect quality and yield of macadamia farms in Levubu, South Africa. PhD Thesis. University of Venda, Thohoyandou.
- Linden, V.M., Grass, I., Joubert, E., Tschamtko, T., Weier, S.M., Taylor, P.J., 2019. Ecosystem services and disservices by birds, bats and monkeys change with macadamia landscape heterogeneity. *J. Appl. Ecol.* 56, 2069–2078.
- Linenberg, I., Christophides, G.K., Gendrin, M., 2016. Larval diet affects mosquito development and permissiveness to *Plasmodium* infection. *Sci. Rep.* 6, 1–10.
- Lorenzen, C.J., 1967. Determination of chlorophyll and pheopigments: spectrophotometric equations. *Limnol. Oceanogr.* 12, 343–346.
- Macadamias South Africa (SAMAC), 2020. An overview of the South African Macadamia Industry [online]. Available from: <https://www.samac.org.za/industry-overview/>. Retrieved 16 October 2021.
- Magbanua, F.S., Townsend, C.R., Blackwell, G.L., Phillips, N., Matthaei, C.D., 2010. Responses of stream macroinvertebrates and ecosystem function to conventional, integrated and organic farming. *J. Appl. Ecol.* 47, 1014–1025.
- Mbare, O., Lindsay, S.W., Fillinger, U., 2014. Pyriproxyfen for mosquito control: Female sterilization or horizontal transfer to oviposition substrates by *Anopheles gambiae* sensu stricto and *Culex quinquefasciatus*. *Parasit. Vectors* 7, 1–12.
- Medeiros, A.O., Pascoal, C., Graça, M.A.S., 2009. Diversity and activity of aquatic fungi under low oxygen conditions. *Freshw. Biol.* 54, 142–149.
- Merritt, R.W., Dadd, R.H., Walker, E.D., 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. *Annu. Rev. Entomol.* 37, 349–374.
- Minakawa, N., Sonye, G., Mogi, M., Yan, G., 2004. Habitat characteristics of *Anopheles gambiae* ss larvae in a Kenyan highland. *Med. Vet. Entomol.* 18, 301–305.
- Morrissey, C.A., Mineau, P., Devries, J.H., Sanchez-Bayo, F., Liess, M., Cavallaro, M.C., Liber, K., 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. *Environ. Int.* 74, 291–303.
- Mutshekwa, T., Cuthbert, R.N., Wasserman, R.J., Murungweni, F.M., Dalu, T., 2020. Nutrient release dynamics associated with native and invasive leaf litter decomposition: a mesocosm experiment. *Water* 12, 2350.
- Muturi, E.J., Allan, B.F., Ricci, J., 2012. Influence of leaf detritus type on production and longevity of container-breeding mosquitoes. *Environ. Entomol.* 41, 1062–1068.
- Muturi, E.J., Donthu, R.K., Fields, C.J., Moise, I.K., Kim, C.H., 2017. Effect of pesticides on microbial communities in container aquatic habitats. *Sci. Rep.* 7, 1–10.
- Muturi, J., Benedict, O.O., Chang-Hyun, K., 2013. Effect of leaf type and pesticide exposure on abundance of bacterial taxa in mosquito larval habitats. *PLoS One* 8, 71812.
- Nortjé, G.P., Botha, A., Schoeman, S.P., Botha, B.M., 2017. The successes of stinkbug research and the Stinkbug Working Group in the Southern African avocado and macadamia industries. South African Avocado Growers' Association Annual Research Symposium. Hotel@Tzaneen, Tzaneen, 15 February 2017.
- Olden, J.D., Kennard, M.J., Leprieux, F., Tedesco, P.A., Winemiller, K.O., García-Berthou, E., 2010. Conservation biogeography of freshwater fishes: recent progress and future challenges. *Divers. Distrib.* 16, 496–513.
- Piscart, C., Genoul, R., Doledec, S., Chauvet, E., Marmonier, P., 2009. Effects of intense agricultural practices on heterotrophic processes in streams. *Environ. Pollut.* 157, 1011–1018.
- Ramos, S.M., Graça, M.A., Ferreira, V., 2021. A comparison of decomposition rates and biological colonization of leaf litter from tropical and temperate origins. *Aquat. Ecol.* 55, 925–940.
- Rasmussen, J.J., Wiberg-Larsen, P., Baatrup-Pedersen, A., Monberg, R.J., Kronvang, B., 2012. Impacts of pesticides and natural stressors on leaf litter decomposition in agricultural streams. *Sci. Total Environ.* 416, 148–155.
- Rossi, F., Pesce, S., Mallet, C., Margoum, C., Chaumot, A., Masson, M., Artigas, J., 2018. Interactive effects of pesticides and nutrients on microbial communities responsible of litter decomposition in streams. *Front. Microbiol.* 9, 2437.
- Saulnier-Talbot, É., Lavoie, I., 2018. Uncharted waters: the rise of human-made aquatic environments in the age of the “Anthropocene”. *Anthropocene* 23, 29–42.
- Schoeman, P.S., 2009. Key biotic components of the indigenous Tortricidae and Heteroptera complexes occurring on Macadamia in South Africa. PhD Thesis. North-West University, Potchefstroom.
- Shurin, J.B., Gruner, D.S., Hillebrand, H., 2006. All wet or dried up? Real differences between aquatic and terrestrial food webs. *Proc. R. Soc. B Biol. Sci.* 273, 1–9.
- Staaaf, H., Berg, B., 1982. Accumulation and release of plant nutrients in decomposing scots pine needle litter. Long-term decomposition in a scots pine forest II. *Can. J. Bot.* 60, 1561–1568.
- Stevenson, R.J., Bothwell, M.L., Lowe, R.L., Thorp, J.H., 1996. *Algal Ecology: Freshwater Benthic Ecosystem*. Elsevier, Cambridge.
- Sumudumali, R.G.I., Jayawardana, J.M.C.K., Gunatilake, S.K., Udayakumara, E.P.N., Malavipathirana, S., Jayalal, R.G.U., 2022. The effects of fungicide chlorothalonil on leaf litter breakdown rate in tropical streams: a microcosm study. *Aquat. Sci.* 84, 1–12.
- Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. *Nature* 467, 555–561.
- Wasserman, R.J., Alexander, M.E., Barrios-O'Neill, D., Weyl, O.L.F., Dalu, T., 2016. Using functional responses to assess predator hatching phenology implications for pioneering prey in arid temporary pools. *J. Plankton Res.* 38, 154–158.
- Zubrod, J.P., Baudy, P., Schulz, R., Bundschuh, M., 2014. Effects of current-use fungicides and their mixtures on the feeding and survival of the key shredder *Gammarus fossarum*. *Aquat. Toxicol.* 150, 133143.