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Soil macrofauna diversity and weed dynamics in response to different methods of weed control in smallholder rubber farming

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Abstract. *Chaniago I, Yulistriani, Umami IM, Bukhari ZZ. 2023. Soil macrofauna diversity and weed dynamics in response to different methods of weed control in smallholder rubber farming. Biodiversitas 24: 3106-3113.* Weeds interference in rubber farmland may reduce latex yield. Farmers apply herbicides to control weeds. However, the herbicide not only controls weed but also affects the presence of soil macrofauna. A study has been conducted to determine the effect of different method of weed control on soil macrofauna and weeds at smallholder rubber farming at Pulau Punjung, Dharmasraya District, West Sumatra, Indonesia. The experiment was conducted in a completely randomized block design with 6 treatments and 4 pseudo-replications. Treatment was mechanical control, without weed control, and 4 doses of herbicide glyphosate+metsulfuron-methyl (1.5 L ha^{-1}+15 g ha^{-1}, 2 L ha^{-1}+15 g ha^{-1}, 2.5 L ha^{-1}+15 g ha^{-1}, 3 L ha^{-1}+15 g ha^{-1}) with 400 L ha^{-1} of volume. Pitfall traps were used to collect the soil macrofauna. Data were analysis with ANOVA for weed and soil macrofauna in response to different methods of weed control. Results demonstrated that the application of glyphosate+metsulfuron-methyl herbicide significantly suppressed weeds higher than that of other methods of weed control (p<0.05). Para grass (*Brachiaria mutica* (Forssk.) Stapf) was the most dominant weed but has been affected most by 3 L ha^{-1}+15 g ha^{-1} herbicide mixture. Soil macrofauna of the order Hemiptera was affected most by the application of herbicide with a total reduction of 71.58% of number of individuals at 12 weeks after application of herbicide. In general, different weed control methods resulted in varied numbers of orders and individuals of the soil macrofauna.

Keywords: Glyphosate, herbicide, metsulfuron-methyl, soil macrofauna, weeds

INTRODUCTION

The rubber plants (*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.) is a plant species whose strategic roles has been recognized worldwide and account for as much as 99% of the world’s natural rubber. It has been planted in more than 40 countries including Indonesia for its latex production (Verheye 2010). However, 85% of Indonesian rubber plantation belongs to small-hold farmers with some complexity and obstacles in managing their farms. The productivity of rubber plants in Indonesia is second to Thailand, i.e., 1.1 and 1.7 ton ha^{-1} $year^{-1}$, respectively (Global Business Guide 2016). The low productivity of rubber plants in Indonesia resulted from various problems, including the age of the trees, the quality of clone seedlings, post-harvest handling, and farming management.

Weeds interfere with crop life and human activities. The economic loss of crops to weed interference has been widely reported, including in maize (Andert 2021; Das et al. 2022; Grzanka et al. 2022; Tyler 2022), oil palm plants (Pasaribu et al. 2017), rubber plants (Wu et al. 2020; Song et al. 2022), sugar cane plantation (Carneiro et al. 2020; Li et al. 2022) and other crops. Weeds have become the main competitor for crops in acquiring resources such as soil nutrients, water, space, and sun light. Furthermore, allelopathic interference may worsen resource competition.

Herbicide has been the most effective mean of weed control that may reduce weeds and improve farmers’ economic returns (Gage et al. 2019; Langaro et al. 2020; Cabrera-Pérez et al. 2022; Chauhan and Mahajan 2022; Fuhrimann et al. 2022). In contrast, the herbicide may have a negative impact on the environment (He et al. 2019; Kwonpongsagoon et al. 2021; Hong et al. 2022) as well as resistance of certain weed species to herbicides such as *Eleusine indica* (L.) Gaertn. resistance to glyphosate at the oil palm plantation (Pasaribu et al. 2017). Herbicide application has also affected non-target species such as earthworms (Kalkhoran et al. 2022), snails (Fadhlaoui and Lavoie 2021), and the colonies’ nutritional dan health status of honey bees (Macri et al. 2021).

A herbicide mixture of two or more active ingredients have commonly been applied to increase the efficacy and broaden the herbicide spectrum (Polli et al. 2021; Dilliott et al. 2022; Gazola et al. 2022; Lajmanovich et al. 2022). Studies demonstrated that the herbicide saflufenacil was added to a mixture of glyphosate + dicamba and applied pre-planting was the most effective to control glyphosate-resistant Canadian horseweed (*Erigeron canadensis* L.). The increase of Canadian horseweed control was reported from 57 to 92, 93 to 99, and 94 to 99% at 2, 4, and 8 Weeks After Application (WAA), respectively (Dilliott et al. 2022). However, another study demonstrated the antagonistic effect of herbicide mixture of metribuzin+halosulfuron and metribuzin+flumioxazin on *Chenopodium album* L., *Amaranthus retroflexus* L. and caused injury in potatoes with a 50% effective dose (ED_{50})

of 1.21, 0.54, and 12.23 g ai·ha^{-1}, respectively (Kalkhoran et al. 2021).

The application of a herbicide, either in sole or a mixture of active ingredients, have negative impacts on the environment, including soil fauna (Zaller and Brühl 2021). A study demonstrated that herbicide application reduced the abundance and community diversities of soil invertebrates in a sugarcane plantation. The author argued that the reduction resulted from either the direct effect of the herbicide or the indirect effects of herbicides in modifying micro-habitat parameters such as weed biomass, amount of mulch, and litter humidity (Coulis 2021). Chemical disturbances due to the intensive use of fertilizer, pesticides, and herbicides may exercise important control on the soil fauna (Cassani et al. 2021) in other ecosystems such as plantain crops (Loranger-Merciris et al. 2022) and conventional land use (Sünnemann et al. 2021). However, another study found that land-use intensification, including the application of agri-chemicals, significantly reduced soil macrofauna biomass. The reduction, interestingly, occurred at the community rather than individual level (Yin et al. 2022).

There is no doubt that long-term application of herbicides results in various effects including weed resistance to certain herbicides, crop phytotoxicity, residual effects in the soil, and harmful effect on non-target species such as soil invertebrates and humans. The effect of various herbicides on non-target species has widely been reported in various cropping systems, such as in sugar cane plantations (Coulis 2021), banana plantations (Loranger-Merciris et al. 2022), and coffee-based agroforestry (Asfaw and Zewudie 2021). However, there is limited information of the effect of herbicide mixtures to control weeds in smallholder rubber farms. Furthermore, the effects of herbicide mixture on soil invertebrates in rubber smallholder farms in West Sumatra have not been reported. Therefore, this study aimed to ascertain the effect of different methods of weed control and their effect on soil macrofauna in smallholder rubber farming.

MATERIALS AND METHODS

Study area

The study was carried out in smallholder rubber (*H. brasiliensis*) farming in a major rubber-growing region in West Sumatra Province, Indonesia and situated at Pulau Punjung, Dharmasraya District (0°56.34831' S and 101°28.78303' E) (Figure 1). This experiment was established in a 14-15 year-old rubber farm from May to August 2021. The annual rainfall is about 249.67 mm and the mean annual air temperature ranges from 20.8 to 34.5°C (BMKG 2021).

Experimental procedures

The experiment was arranged in a completely randomized block design with six treatments and four pseudo-replications. The treatment was different methods of weed controls as follows: Doses of herbicide mixture of glyphosate and metsulfuron-methyl (1.5 L ha^{-1}+15 g ha^{-1} (A); 2 L ha^{-1}+15 g ha^{-1} (B); 2.5 L ha^{-1}+15 g ha^{-1} (C); and 3 L ha^{-1}+15 g ha^{-1} (D)), Mechanical weed Control (MC) using a scythe, and without weed control (NC). The use of the herbicide dose was within the range of recommended dose for glyphosate that is 1.5-4.3 L ha^{-1} and 7-20 g ha^{-1} for metsulfuron-methyl. The herbicide was applied at the volume of 400 L ha^{-1}. The size of each experimental unit was 15 m x 2 m.

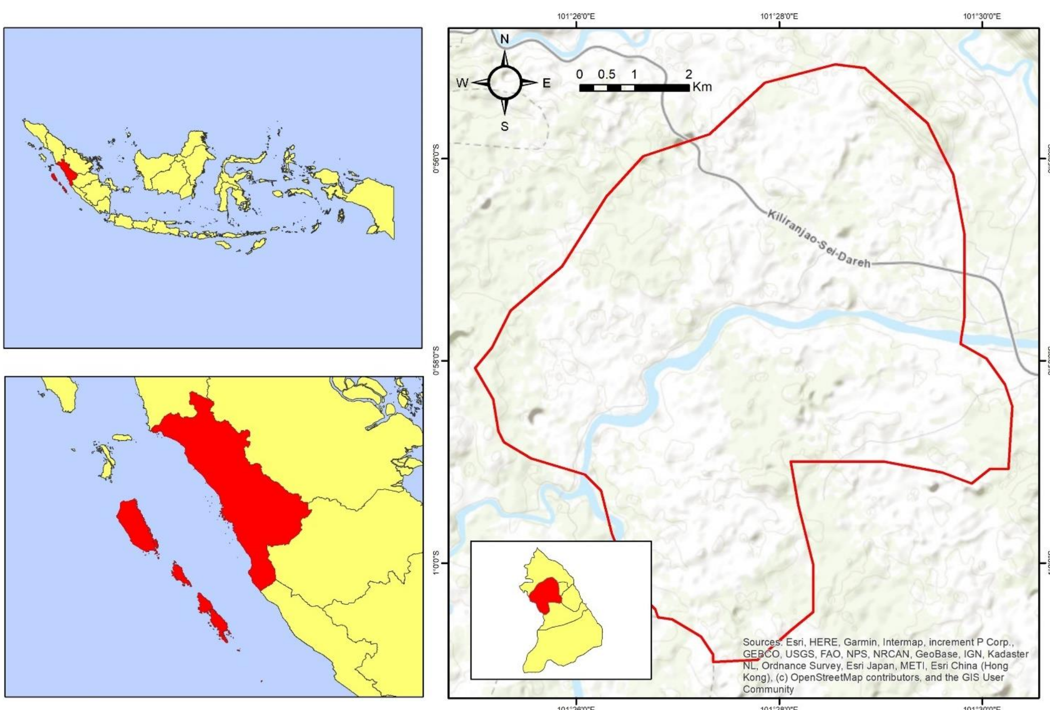

Figure 1. Map of experimental site of smallholder rubber farming at Pulau Punjung, Dharmasraya District, West Sumatra, Indonesia

Weed collection

Weeds were collected from two sub-plots. Each sub-plot of 50 cm x 50 cm was prepared in every experimental unit for every sampling time. The sampling was conducted at one week before herbicide application (1 WBA) and at 4, 8, and 12 Weeks After Application (WAA). Weeds were carefully extracted from the soil, then cleaned, identified, counted, cut, and hot-air dried at 80°C for 48 hours and weighed for calculating weed biomass and SDR values. Weeds were also collected from the No weed Control (NC) and Mechanical weed Control (MC) treatment groups at the same sampling times as for herbicide treatment groups. Weeds were identified according to weed manual book identification (Naidu 2012; Sriyani et al. 2014).

Herbicide application

Prior to herbicide application, the calibration was carried out using a knapsack sprayer so that each experimental unit received a similar amount of herbicide according to treatment groups. Each experimental unit needed 1.2 L of herbicide solution. The herbicide was applied on the morning of a clear day starting with the lowest dose upward.

Macrofauna sampling

Soil macrofauna was collected using pitfall trap which was installed for 2 units and was distanced for 8 m from one another in every experimental unit. Plastic cups of 250 mL size filled with 75 mL of propylene glycol, were buried in the soil and used to trap the soil macrofauna. The pitfall traps were installed 1 Week Before herbicide Application (1 WBA) for collecting soil macrofauna. Then, the consecutive installations were conducted at 3, 7, and 11 Weeks After Application (WAA) of herbicide treatment and were maintained for one week prior to further collecting of soil macrofauna. The time of soil macrofauna collection was similar to that of weed collection (4, 8, and 12 WAA).

Data analysis

Weed dominance was demonstrated as a Summed Dominance Ratio (SDR) value and was calculated (Janiya and Moody 1989). The diversity factor of soil macrofauna such as Shannon-Weinner Diversity Index (H’) and Shannon evenness Index (E) was determined (Krebs 1999) to estimate population dynamics. Data of para grass biomass reduction and soil macrofauna were subjected to analysis of variance and mean separation by Duncan’s New Multiple Range Test (significance level $p<0.05$) with computer software of STAR®.

The diversity and evenness indices of soil macrofauna was calculated as follow:

$$H' = -\sum_{i=1}^{R} pi \ln pi$$

H’ : Index of diversity
R : Number of individuals of macrofauna
p_i : Proportion of individual species to total population
ln : Logarithm natural

$$E = H'/ln\ S$$

E : Index of evenness
H’ : Index of diversity
S : Number of species found
Ln : Logarithm natural

RESULTS AND DISCUSSION

Weed shifting

Herbicide application in rubber plants farming affected weeds associated with the rubber plants. There was 8 weed species identified at the experimental site prior to herbicide application. The weeds are *Brachiaria mutica* (Forssk.) Stapf, *Scleria sumatrensis* Retz., *Nephrolepis biserrata* Schott, *Asystasia gangetica* (L.) T.Anderson, *Carex muskingumensis* Schwein, *Axonopus compressus* (Sw.) P.Beauv., *Paspalum conjugatum* P.J. Bergius, and *Clidemia hirta* (L.) D. Don with the value of Summed Dominance Ratio (SDR) of 72.6, 7.3, 7.8, 4.5, 1.2, 1.3, 1.8, and 3.3%, respectively. Para grass (*B. mutica*) was found to be the most dominant weed species at the experimental site with a SDR value of 72.6%. However, significant reduction ($p<0.05$) of para grass biomass was recorded at 4, 8, and 12 WAA in all herbicide treatment groups (Figure 2). An increase in herbicide dose was followed by a significant reduction in weed biomass at 4 WAA of herbicide.

The highest reduction in weed suppression indicated by the lowest amount of weed biomass had been observed from the 3 L ha^{-1}+15 g ha^{-1} combined herbicide glyphosate+metsulfuron-methyl treatment group. The eradicated weed changes the moisture and temperature of the soil surface which in turn affects the activity of soil microbes (Peña-Venegas et al. 2021; Tall and Puigbò 2022), enzymes (Chaudhary et al. 2021; Tyler 2022), and macrofauna (Lacava et al. 2021). Weeds have been known as an alternate host for some pests and pathogens; hence changes in weed population results in changes in some soil macrofauna. The herbicide mixture was more effective in controlling weeds than the sole herbicide for controlling Bermuda grass in maize cultivation (Chipomho et al. 2020), damaged chloroplast, mitochondria, and nutritional value in *Phaeodactylum tricornutum* Bohlin, 1897 (Wang et al. 2022). For some of these reasons and higher efficacy, farmers prefer to apply a mixture of herbicides to sole herbicides.

The mixture of herbicides is aimed at enhancing the spectrum of herbicide efficacy and has been successfully used in various agricultural ecosystems (Carneiro et al. 2020; Vidotto et al. 2022; Wang et al. 2022). For instance, the mixture of Glyphosate at 1080 g ha^{-1} and paraquat at 600 g ha^{-1} has completely controlled navua sedges (*Cyperus aromaticus* (Ridl.) Mattf. & Kük.) (Chauhan and Mahajan 2022). The mixture herbicide glyphosate and metsulfuron-methyl in the experiment reported here has successfully controlled all weed species. Para grass was affected the most. All herbicide treatments resulted in higher weed biomass reduction compared to the treatment group of mechanical control or no weed control. Four WAA of the herbicide treatment, *B. mutica* died and may alter the soil surface condition. These demonstrated the effectiveness of the combined herbicide in controlling the weed (Figure 3). Glyphosate herbicide is known as a broad-spectrum systemic herbicide and crop desiccant. Glyphosate inhibits enzyme 5-enolpyruvylshikimate-3-phosphate synthase as well as acts as a desiccant. This desiccating property may enhance efficacy through rapid loss of plant water content. On the other hand, metsulfuron-methyl affects weed through the inhibition of Acetolactate Synthase (ALS) and Acetohydroxyacid Synthase (AHAS) enzymes (Yu et al. 2021).

Soil macrofauna

Combined herbicide treatment of glyphosate and metsulfuron-methyl significantly affected soil macrofauna at the rubber farming (p<0.05). The Order Hemiptera was affected most at all observation times in response to the treatments (Figure 4, I-IV). The reduction outnumbered other orders in all time of observations in response to weed control.

There were four orders of soil macrofauna found to be dominant at the experimental site prior to weed control. They were Orthoptera, Hymenoptera, Hemiptera, and Arachnida. However, weed control significantly reduced the individual number of Hemiptera (p<0.05). The reduction was observed from 4 to 12 WAA of herbicide. The total amount of soil macrofauna of the Order Hemiptera was 278 individuals prior to herbicide application and was reduced by as much as 71.58% at 12 WAA of herbicide. There was no individual of soil macrofauna of Hemiptera found from the highest dose of combined herbicide treatment group (3 L ha^{-1}+15 g ha^{-1}) at 12 WAA of herbicide. Most Hemipteran animals feed on plants using their sucking and piercing mouthparts (Kumara et al. 2021). When the plants dried and died, this order will lose their feed due to unavailability of the plant sap to be sucked. They may have moved to another place and seek for other feed. The orders of Coleoptera and Arachnida were affected the least by different weed control including herbicide mixture application. A slight reduction in the individual number of Arachnida was observed at 4 WAA of herbicide.

On the other hand, orders of Stylommatophora, Oligochaeta, and Dermaptera were consistently lowest in the number of soil macrofauna under the experimental conditions. The land snails of Stylommatophora do not move far over their lifetime and are sensitive to calcium availability for their shell formation and development (Zuliantoni et al. 2022). The small number of land snail found in this experiment could be an indicator that the soil is low in calcium (Sari 2021) with pH of 4.6 and low in N, P, and K. The earthworm of the order of Oligochaeta is mostly burrow into the soil and result in a very small amount of individual found in the trap during this experiment. Earthworm has an avoidance behavior towards pesticides (Yang et al. 2018). These favor the earthworm from the negative effects of pesticides applied at the farm. We found less than 10 individuals of the order Dermaptera at 12 WAA of herbicide. Dermaptera are classified as nocturnal insects. During the day, they usually hide in cracks and crevices, under the bark of trees, or in rubbish on the ground (Gibb 2015).

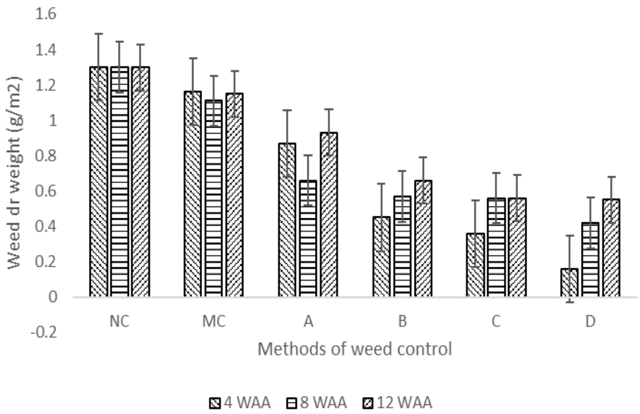

Figure 2. Biomass (g m^{-2}) of *B. mutica* weed in response to different weed control. Notes: NC: No weed Control, MC: Mechanical weed Control, A: 1.5 L ha^{-1}+15 g ha^{-1}, B: 2 L ha^{-1}+15 g-ha^{-1}, C: 2.5 L ha^{-1}+15 g-ha^{-1}, and D: 3 L ha^{-1}+15 g ha^{-1} combined herbicide glyphosate+metsulfuron-methyl, respectively. Bars: standard error

Figure 3. *Brachiaria mutica* weed. A. Prior to application of combined herbicide of glyphosate and metsulfuron-methyl, B. Deceased *B. mutica* at 4 WAA of 3 L ha^{-1}+15 g ha^{-1} combined herbicide treatment

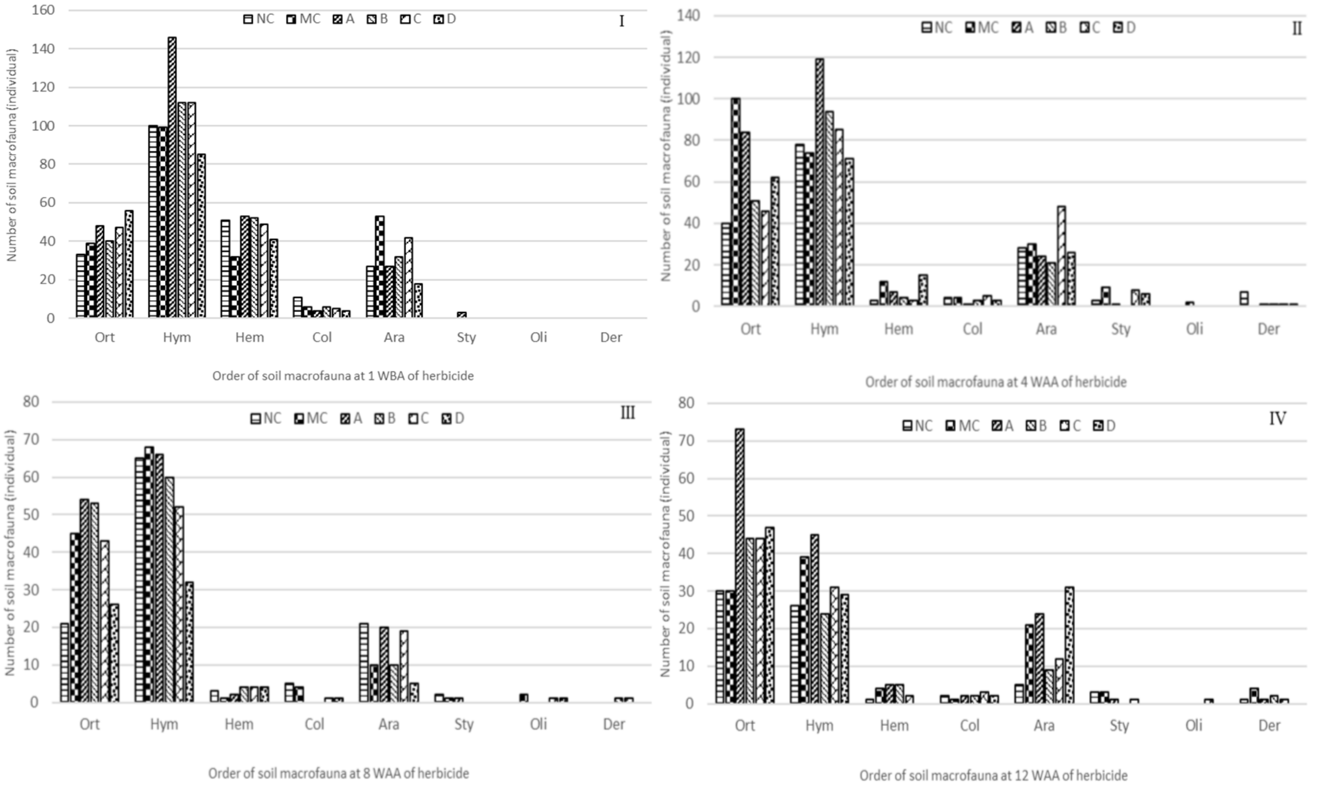

Figure 4. The amount of soil macrofauna in response to combined herbicide of glyphosate and metsulfuron-methyl. Notes: NC: No weed Control, MC: Mechanical weed Control, A: 1.5 L ha^{-1}+15 g ha^{-1}, B: 2 L ha^{-1}+15 g ha^{-1}, C: 2.5 L ha^{-1}+15 g ha^{-1}, and D: 3 L ha^{-1}+15 g ha^{-1} combined herbicide of glyphosate and metsulfuron-methyl. Ort: Orthoptera, Hym: Hymenoptera, Hem: Hemiptera, Col: Coleoptera, Ara: Arachnida, Sty: Stylommatophora, Oli: Oligochaeta, Der: Dermaptera

Table 1. Changes in soil macrofauna in response to different means of weed control at smallholder rubber farming

Observation	1 WBWC*	Treatments**					
		NC	MC	A	B	C	D
Number of orders	5	7	7	8	6	8	7
Number of soil macrofauna	1433	68	102	151	86	95	109
Diversity index	1.46	1.29	1.45	1.24	1.28	1.32	1.15
Evenness index	0.78	0.66	0.74	0.64	0.66	0.68	0.59

Note: *: Total data of soil macrofauna collected from all sampling sites for all treatment groups, **: Data of soil macrofauna collected at 12 weeks after herbicide application of different treatment groups. WCBC: Week Before weed Control, MC: Manual weed Control, NC: No weed Control, A: 1.5 L ha^{-1} + 15 g ha^{-1}, B: 2 L ha^{-1} + 15 g ha^{-1}, C: 2.5 L ha^{-1} + 15 g ha^{-1}, D: 3 L ha^{-1} + 15 g ha^{-1}. A, B, C, D are treatments of herbicide mixture of glyphosate + metsulfuron-methyl according to tested dose

The variation in soil macrofauna found at different collection times is not surprising. The soil macrofauna was very mobile and actively sought their feed, such as plant litter (Musyafa 2005) from their surroundings. Weed control that resulted in changes in the vegetation height might have altered the feeding habits of the soil macrofauna (Rebrina et al. 2022). The variation of macrofauna in response to herbicide application highlighted their movement to a different location where the feed is available, so that some macrofauna might decrease (Sundar et al. 2021). The applied herbicide has changed the vegetation and directly affected the number and diversity of soil macrofauna. Phytophagous was affected the most. Table 1 demonstrates changes in the population dynamics of the soil macrofauna due to weed control under experimental conditions.

The population dynamics of soil macrofauna varied in response to weed control methods. The variation in the number of soil macrofauna might have resulted from the disturbed floral composition due to weed control. The lowest amount of soil macrofauna was observed at the no-weed-control treatment at 12 WAA of herbicide. This might be due to non-disturbed ecosystem where they live with living grass and other weeds. No or minimum disturbance will not affect the soil macrofauna. The changes in floral composition affected the macrofauna as they are more active in a slightly opened soil surface (Vazquez et al. 2020; Kelly et al. 2021). Weed control changed the soil macrofauna from a stable community ($0.75 < E \leq 1$) to an unstable community with an index value of 0.59-0.74. These changes demonstrated the disturbance in the soil macrofauna community in response to weed control. The highest dose of herbicide mixture (3 L ha^{-1} + 15 g ha^{-1}) resulted in the lowest index of evenness (0.59). The soil macrofauna diversity index was slightly reduced by weed control. However, the treatments did not change the category of their existence which is low in diversity. Our finding is in accordance with the work of others who reported that agrichemical application affected soil fauna communities (Niemeyer et al. 2018; Asfaw and Zewudie 2021; Coulis 2021). Abiotic and biotic factors, including feed availability and natural enemies (predators), affected the variation of diversity and distribution of soil macrofauna. Changes in coverage and height of vegetation due to herbicide application resulted in reduced amounts of soil macrofauna. The unstable community and low diversity of soil macrofauna may decelerate organic matter decomposition (Cassani et al. 2021) under the shade of rubber trees. Therefore, keeping biodiversity in the agricultural ecosystem would promote balance in food chains.

Agrichemicals may shift the vegetation whose presence are important for the existence of other species (Comeau and Fraser 2018; Deligios et al. 2019), including soil macrofauna. Excessive use of herbicides affects plant and animal performance and may influence trophic interactions such as herbivory and pollination (Ruuskanen et al. 2023). These animals contribute to the balance in an ecosystem. Another study found that an herbicide mixture of 2,4-D and glyphosate significantly decreased the fertilization and the oocytes and the hatchery of the eggs and damaged the genetic material of larvae of silver catfish (Bernardi et al. 2022). Similarly, the mixture of herbicide glyphosate and glufosinate ammonium caused ecotoxicity in anuran tadpoles (Lajmanovich et al. 2022). These reports have demonstrated the effects of herbicide mixture in situ. The downstream effects of the herbicide may occur through the water flow and may affect the quality of irrigation for farming.

The presence and diversity of soil macrofauna are closely related to the health of the soil where they live and result from their ability to promote litter decomposition (Lin et al. 2019; Cassani et al. 2021). Land conversion from natural forest to crop plantation followed by extensive use of agrochemicals has lowered soil health through changes in vegetation structure, litter biomass, and ground vegetation cover (Rohyani and Ahyadi 2017; Nguyen et al. 2020) and a decrease in ecosystem services (Arunyawat and Shrestha 2016). These changes will, in turn, affect the presence and livelihood of soil macrofauna. Another work demonstrated that the application of glyphosate herbicide at 200 µg L^{-1} increased Glutathione-S-Transferase (GST) in snails (*Lymnaea* sp.) indicating the detoxification of glyphosate (Fadhlaoui and Lavoie 2021). This report suggested that soil invertebrates possess of detoxifying mechanism to resist the effect of agri-chemicals exposure. It is interesting to note that soil macrofauna and aerial invertebrate such as stingless bee has been non-target species affected by the herbicide. Glyphosate was very toxic to the bee larvae and killed them a few days after exposure (Seide et al. 2018). Another study demonstrated that feeding with a solution containing 2.5, 5, and 10 mg L^{-1} (sublethal concentrations) of glyphosate affected the homeward flying path of honeybees. The highest concentration resulted in more time needed by the honeybees to return to the apiary (Balbuena et al. 2015).

Our results have confirmed that herbicide application and manual weed control may affect non-target species, including soil macrofauna. Para grass was the most dominant weed but has been affected most by 3 L ha^{-1} + 15 g ha^{-1} herbicide mixture of glyphosate and metsulfuron-methyl. All methods of weed control affected the population dynamics of the soil macrofauna under the experimental conditions. The highest total reduction of 71.58% at 12 WAA of herbicide was observed in Order Hemiptera. Precautionary measures should be taken into consideration when applying herbicides to minimize negative impacts on the environment and non-target species.

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