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Weed diversity and microbial responses to glyphosate dissipation in amended coffee soils

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Abstract. *Rismayanti AY, Sumekar Y, Widayat D, Yuwariah Y, Murtilaksono A. 2026. Weed diversity and microbial responses to glyphosate dissipation in amended coffee soils. Biodiversitas 27 (1): d270144. https://doi.org/10.13057/biodiv/d270144.* Glyphosate dissipation in tropical coffee soils is an important ecological concern because residue dynamics may influence weed communities and soil microbial activity. Understanding how soil amendments modify these responses is essential for strengthening sustainable weed management in perennial crops. This study evaluated the effects of biochar and manure on glyphosate dissipation, weed dynamics, and microbial respiration in Arabica coffee soils. A split-plot field experiment with three replications was conducted, combining two organic amendments and six glyphosate doses; weed biomass was analyzed using ANOVA, while glyphosate residues and soil microbial respiration were evaluated descriptively using composite samples. Before herbicide application, 19 weed species were recorded, dominated by *Panicum repens*, *Cynodon dactylon*, and *Amaranthus spinosus*. Following glyphosate application, weed diversity temporarily declined from H′ = 2.27 to 2.12 at 14 days after application (DAA) and subsequently recovered to 2.29-2.33 by 28-42 DAA, while community similarity remained high (>97%). Weed biomass was significantly affected by glyphosate dose at all observation times, with a significant interaction between glyphosate dose and soil amendment detected only at 28 DAA. Organic amendments, particularly biochar and manure, were associated with lower weed biomass and a less pronounced decline in weed diversity during the early phase of glyphosate application. Amended soils showed higher measured microbial respiration compared with unamended soils during the observation period. Glyphosate residue concentrations showed a faster apparent decline in amended soils, with lower measured values observed by 42 days after application. These findings indicate that organic materials are associated with differences in glyphosate dissipation and soil microbial respiration patterns. However, glyphosate residue and microbial respiration data were derived from non-replicated composite samples; therefore, observed trends should be interpreted cautiously. The use of organic amendments, particularly biochar, was associated with changes in weed biomass, microbial respiration patterns, and glyphosate dissipation in coffee soils, indicating their potential relevance for integrated and environmentally conscious weed management.

Keywords: *Coffea arabica*, glyphosate dissipation, organic amendments, soil ecology, weed diversity

INTRODUCTION

Indonesia, one of the highest coffee-producing countries worldwide, significantly influences the global coffee market. The Arabica coffee agroecosystem has been evolving, with the presence of weeds and soil microorganisms contributing to both productivity and ecosystem sustainability (Archibald et al. 2022). Weeds influence both competition and ecological stability in coffee plantations, while soil microorganisms regulate nutrient cycling and organic matter turnover. The interaction between weed communities and soil microbial processes contributes to long-term ecosystem functioning in coffee-based agroecosystems. To manage weed competition while maintaining the stability of soil microbial communities, coffee farmers often rely on herbicides such as glyphosate.

Most coffee farmers utilize glyphosate-based herbicides to control weeds because of their practical application and high effectiveness (Casimero et al. 2022; Istriningsih et al. 2022). Glyphosate application is widely associated with effective weed suppression through interference with plant metabolic processes. Long-term glyphosate application is associated with weed diversity transformation, certain species domination, and residue emergence in soil (van Bruggen et al. 2021).

Moreover, the proliferation of glyphosate-resistant weed species, exemplified by *Ageratum conyzoides* L. and *Eleusine indica* (L.) Gaertn., has been documented across numerous tropical agricultural systems, signifying the selective pressures arising from the continual application of herbicides (Kurniadie et al. 2023). Furthermore, glyphosate is known to have a negative impact on soil microbes through its presence as residues in soil environments, which may be associated with changes in soil biological functioning. Glyphosate, while efficient for perennial weed suppression, poses ecological risks due to residue accumulation and microbial imbalance. To address these concerns, organic soil amendments such as biochar and manure have been increasingly explored as management options.

Understanding glyphosate dissipation under organically amended soils is essential for developing sustainable weed management strategies. Recent studies have demonstrated that microbial populations are associated with changes in glyphosate residue dynamics, yet their behavior under biochar- and manure-amended coffee soils remains unclear. This knowledge gap limits integrated evaluation of glyphosate use, weed community responses, and soil biological indicators in coffee agroecosystems.

The utilization of organic materials, like biochar and manure, has been considered as a strategy to reduce the negative impact of herbicides on soil (Obregón Alvarez et al. 2021). Biochar and manure are reported to modify soil physical and chemical properties and to be associated with differences in glyphosate dissipation through changes in soil conditions. A combination of the application of herbicides and soil organic matter becomes an important approach in controlling weeds, along with soil ecological maintenance (Twagirayezu et al. 2024). However, studies that simultaneously evaluate weed diversity, glyphosate dissipation, and soil microbial respiration within a single experimental framework remain limited.

Existing studies address these components separately, leaving uncertainty about how organic amendments influence glyphosate dissipation and ecological responses in an integrated manner. Most studies focus primarily on agronomic or soil chemical aspects, while comprehensive evaluations related to weed diversity dynamics and soil microbial respiration under glyphosate exposure or organic matter are still rarely undertaken. Such integrative information is important for environmentally responsible weed management and long-term soil health maintenance.

Based on these considerations, this study was conducted to evaluate weed diversity, weed biomass, glyphosate dissipation, and soil microbial respiration in Arabica coffee agroecosystems under glyphosate and organic amendment treatments. This study aimed to evaluate the combined effects of glyphosate dose and organic amendments on weed biomass using inferential statistics, and to explore indicative patterns of weed diversity, glyphosate dissipation, and soil microbial respiration under amended coffee soils. This study is expected to provide a more detailed understanding of the impact of herbicides on biodiversity and soil ecological processes, and to serve as a basic knowledge for developing sustainable coffee agroecosystem management practices.

MATERIALS AND METHODS

Research location

This study was conducted in an Arabica coffee plantation in Tanjung Karya Village, Garut District, West Java, Indonesia, in 2024, at ±1,200 m asl. Daily temperature was observed at 17-24°C, with a relatively high humidity level. The study site represents a typical highland Arabica coffee agroecosystem in volcanic regions of Indonesia, providing a relevant field context for evaluating glyphosate dissipation and associated weed and soil microbial responses under tropical coffee cultivation conditions. The soil was characterized as an andisol soil order, with a sandy clay texture.

Procedures

Initial soil analysis

Before applying the treatments, a soil sample was taken from the study location to determine its chemical characteristics, including pH, organic-C, total-N, available-P, K-dd, and cation exchange capacity (CEC) (Table 1). A preliminary soil analysis was conducted before applying treatments to the coffee fields, covering key chemical parameters. The dominant microbes were isolated from rhizosphere soil, identified based on colony reflections, and those most relevant to herbicide biodegradation and soil health were selected; therefore, microbial composition results are interpreted descriptively. This analysis was performed in the Laboratory of Plant Chemistry and Nutrition, Faculty of Agriculture, Universitas Padjajaran.

Experimental design and treatments

This research uses a split-plot design in a completely randomized block with three replications. The main plot factor consists of three types of soil amendments (B): no amendment, 10 t ha^{-1} manure, and 10 t ha^{-1} biochar. The sub-plot factor consists of six doses of glyphosate (G): 0, 1.50, 2.25, 3.00, 3.75, and 4.50 L ha^{-1}. The complete combination of both factors is presented in Table 2.

To clarify the experimental layout, the study consisted of three blocks (replications), each representing a relatively homogeneous area of the coffee field. Within each block, soil amendment treatments were randomly assigned to the main plots. Each main plot was subdivided into six subplots, to which glyphosate doses were randomly allocated. Consequently, each block contained 18 experimental subplots (3 soil amendments × 6 glyphosate doses), resulting in a total of 54 experimental units. Randomization was conducted independently within each block to minimize spatial variability.

Each experimental plot measured 3 × 5 m with a coffee planting distance of ± 2 × 2 m. Soil amendment was applied one month before glyphosate spraying to ensure optimal incubation before herbicide treatment. Biochar and goat manure were applied at a rate of 10 t ha^{-1} as soil amendments. The applied materials were locally sourced to reflect commonly available organic amendments in coffee agroecosystems. Amendments were characterized only to provide general descriptive information; interpretations were based on observed field responses rather than detailed physicochemical mechanisms. Glyphosate was applied once using a low-pressure sprayer.

Due to methodological and logistical constraints, inferential statistical analysis in this study was applied only to weed biomass data obtained from replicated experimental units. Weed diversity indices, glyphosate residue concentrations, soil microbial respiration, and dominant microbial identification were based on composite or non-replicated samples and were therefore intended to describe site-level and temporal patterns rather than to test treatment effects statistically.

Table 1. Initial soil characteristics and dominant microbes in coffee experimental land

Initial doil parameters	Value	Unit	Dominant microbes	Abundance (CFU/g soil)
pH (H_2O)	5.6	-	*Pseudomonas* sp.	1.47×10^{11}
Organic-C	2.1	%	*Bacillus* sp.	7.74×10^{9}
Total-N	0.21	%	-	-
Available-P (Bray)	18.4	ppm	-	-
K-dd (potassium level)	0.42	cmol(+)/kg	-	-
Cation exchange capacity	18.5	cmol(+)/kg	-	-
Texture	Clayey loam	-	-	-

Table 2. Soil amendment and glyphosate treatments

Code	Treatment
b0g0	No amendment + 0 L ha^{-1} glyphosate
b0g1	No amendment + 1.50 L ha^{-1} glyphosate
b0g2	No amendment + 2.25 L ha^{-1} glyphosate
b0g3	No amendment + 3.00 L ha^{-1} glyphosate
b0g4	No amendment + 3.75 L ha^{-1} glyphosate
b0g5	No amendment + 4.50 L ha^{-1} glyphosate
b1g0	10 t ha^{-1} goat manure + 0 L ha^{-1} glyphosate
b1g1	10 t ha^{-1} goat manure + 1.50 L ha^{-1} glyphosate
b1g2	10 t ha^{-1} goat manure + 2.25 L ha^{-1} glyphosate
b1g3	10 t ha^{-1} goat manure + 3.00 L ha^{-1} glyphosate
b1g4	10 t ha^{-1} goat manure + 3.75 L ha^{-1} glyphosate
b1g5	10 t ha^{-1} goat manure + 4.50 L ha^{-1} glyphosate
b2g0	10 t ha^{-1} biochar + 0 L ha^{-1} glyphosate
b2g1	10 t ha^{-1} biochar + 1.50 L ha^{-1} glyphosate
b2g2	10 t ha^{-1} biochar + 2.25 L ha^{-1} glyphosate
b2g3	10 t ha^{-1} biochar + 3.00 L ha^{-1} glyphosate
b2g4	10 t ha^{-1} biochar + 3.75 L ha^{-1} glyphosate
b2g5	10 t ha^{-1} biochar + 4.50 L ha^{-1} glyphosate

Soil sampling

The soil sample, taken from the 0-20 cm layer at several time intervals (0, 7, 14, 28, and 42 days), was analyzed to determine the glyphosate residue, microbial respiration, and dominant microbes' identification. For glyphosate residue and microbial respiration analyses, soil samples were composited across plots within each treatment and sampling time by combining three sub-sampling points, resulting in one composite sample per treatment per observation time. Samples were stored under cold conditions (4°C) and promptly transported to the laboratory for further analysis.

Glyphosate residual analysis

Glyphosate residues were quantified using High Performance Liquid Chromatography (HPLC, Shimadzu LC-20AT) equipped with a C18 column (250 mm × 4.6 mm). Detection was performed at 195 nm with a mobile phase of 0.1 M KH_2PO_4 and methanol (60:40, v/v) at 1 mL min^{-1}. The limit of detection (LOD) was 0.01 mg L^{-1}. Calibration curves were prepared using five concentration levels ($R^2 > 0.998$). Soil extraction was carried out using a phosphate buffer solution, followed by filtration and injection into the HPLC system. Residue measurements were conducted using composite soil samples without replication due to analytical cost and instrument-access limitations. Consequently, residue values are presented descriptively and should be interpreted as indicative rather than statistically inferential measurements. This non-replicated sampling approach is a methodological limitation. Therefore, glyphosate residue data were used only to describe temporal dissipation patterns and were not analyzed inferentially, since composite samples did not provide plot-level replication.

Microbial respiration and dominant microbe identification

Microbial respiration in soil was measured at the Laboratory of Soil Biology, Faculty of Agriculture, Universitas Padjadjaran, using the NaOH-trap method. A 100 g soil sample was incubated in a sealed container with 0.05 N NaOH solution as a CO_2 absorbent. After 7 days of incubation at room temperature, the amount of CO_2 was determined by HCl titration, followed by $BaCl_2$ precipitation. A blank control (container without soil) was included, and CO_2 trapped in the blank was subtracted from each sample to correct for background CO. The amount of CO_2 evolved was converted to CO_2-C using the 12/44 molecular weight factor before normalization to soil dry weight. Respiration results were expressed in mg CO_2-C per kg of soil per day.

Dominant microbes were identified on soil samples from each treatment. Microbes were isolated through serial dilution and poured on selective media. Dominant colonies were then morphologically identified to provide a general overview of the soil microbial community associated with glyphosate treatment and organic amendments. Dominant colonies were characterized morphologically (Gram staining, colony color, and texture), acknowledging that molecular confirmation (16S rRNA sequencing) was not performed due to facility constraints. Hence, microbial identification was restricted to the morphological level, and results were used primarily to indicate the presence of dominant microbial groups associated with glyphosate-treated and amended soils. Microbial respiration measurements were derived from the same composite, non- replicated soil samples. Microbial identification relied on colony morphology and was performed on a non-replicated composite sample; thus, the results should be interpreted as indicative rather than conclusive.

This morphological approach does not allow reliable taxonomic resolution at the species or genus level and cannot confirm specific microbial roles in glyphosate degradation. Therefore, microbial data in this study were intended to provide a descriptive overview of dominant microbial groups associated with the treatments, rather than to infer specific taxonomic identities or mechanistic pathways.

Weeds vegetation analysis

Baseline weed vegetation analysis was conducted once prior to soil amendment application and glyphosate spraying to characterize the initial weed community structure. Weed sampling was conducted using a 0.5 × 0.5 m quadrat randomly placed at three points in each treatment plot. All weeds within the quadrat were identified to the species level. This baseline vegetation data were used to calculate weed dominance, diversity indices (H´, D, E), community similarity, and spatial distribution patterns in a descriptive manner. Repeated vegetation composition analysis was not conducted after herbicide application; subsequent observations focused exclusively on weed biomass dynamics across observation times. Weed dry biomass was measured at 0, 14, 28, and 42 days after application (DAA) and constituted the only dataset subjected to inferential statistical analysis. All weed diversity, dominance, community similarity, and spatial pattern metrics were analyzed quantitatively but interpreted descriptively, without testing treatment effects or temporal differences statistically.

Data on weed density, frequency, and dry weight were used to calculate Relative Density (RD), Relative Frequency (RF), Relative Dominance (RDo), and the Summed Dominance Ratio (SDR) (Kurniadie et al. 2020), using the following formulas:

Relative Density (RD):
RD (%) = (Absolute density value of one species / Total absolute density value of all species) × 100

Relative Frequency (RF):
RF (%) = (Absolute frequency value of one species / Total absolute frequency value of all species) × 100

Relative Dominance (RDo):
RDo (%) = (Absolute dominance value of one species / Total absolute dominance value of all species) × 100

Importance Value and Summed Dominance Ratio (SDR):
SDR (%) = (RD + RF + RDo) / 3

The species with the highest SDR value was considered the dominant weed in the community.

Diversity Indices were calculated using the Shannon–Wiener diversity index (H′), Simpson dominance index (D), and Evenness index (E) following Barbour et al. (1987):

Diversity index (H′):

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

Dominance index (D):

$$D = \sum_{i=1}^{s} p_i^2$$

Evenness index (E):

$$E = \frac{H'}{\ln S}$$

Where: Pi = ni/N, ni: number of individuals of species *i*, N: total number of individuals of all species, S: total number of weed species.

Community similarity between observation times was determined using the formula of Tjitrosoedirdjo et al. (1984):

$$C = \frac{2W}{a + b} \times 100\%$$

Where W is the sum of the lower number of shared species between two communities, and a and b are the total number of individuals in the first and second community, respectively.

Spatial distribution patterns were determined using the ratio between the standard deviation (SD) and the mean ($\bar{X}$):

$$\frac{SD}{\bar{X}} = \begin{cases} < 1, & \text{Regular distribution} \\ = 1, & \text{Random distribution} \\ > 1, & \text{Clumped distribution} \end{cases}$$

Weed biomass was measured at 0, 7, 14, 28, and 42 DAA. Weeds within each quadrat were harvested, oven-dried at 80°C until constant weight, and weighed. Dry biomass data were used to evaluate glyphosate effectiveness under each combination of organic amendment and herbicide dosage.

Data analysis

Data were analyzed using a split-plot analysis of variance (ANOVA), with soil amendment as the main-plot factor and glyphosate dose as the subplot factor. Inferential statistical analysis was applied only to weed dry biomass data obtained from replicated experimental units. Treatment effects were tested using the F-test at a 5% significance level, and mean separation was conducted using the Least Significant Difference (LSD) test at the same probability level. Prior to ANOVA, data were examined for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test to ensure compliance with ANOVA assumptions. All inferential statistical analyses were performed using SPSS version 25.0.

Weed diversity indices, dominance values, and weed spatial distribution patterns were calculated based on observations from replicated plots but were interpreted descriptively to illustrate community-level trends rather than subjected to inferential statistical testing. Glyphosate residue concentrations, soil microbial respiration, and dominant microbial identification were obtained from non-replicated composite soil samples and were therefore analyzed descriptively. These datasets were used to describe temporal patterns and treatment-associated tendencies without statistical inference. Inferential statistics were restricted to replicated weed biomass data, whereas weed diversity indices, glyphosate residues, microbial respiration, and dominant microbial groups were analyzed descriptively to illustrate site-level and temporal patterns.

RESULTS AND DISCUSSION

Initial condition of weed vegetation composition

Before herbicide application, the weed community structure in Arabica coffee plantations must be understood, as strongly influenced by interactions among coffee canopy shading, maintenance practices (planting distance, pruning, and mulching), and site-specific soil properties, including pH, organic matter content, and cation exchange capacity (Waheed et al. 2022; Yousaf et al. 2022). The study site was located at approximately 1,200 m above sea level, with daily temperatures measured at 17-24°C and relatively high humidity. The soil is characterized as an Andisol order, with a sandy clay texture, pH of 5.6, and low to moderate organic carbon content (~2.1%). These site characteristics provide the environmental context under which the initial weed composition was observed (Chaniago et al. 2023; Lebbink and Fensham 2023). Organic amendments, namely biochar and manure, were applied a month before spraying, thus initially altering the physicochemical conditions and microbial habitat in soil.

Table 3 presents the composition of weed vegetation prior to glyphosate application. A total of 19 weed species were recorded, with three species, *Panicum repens*, *Cynodon dactylon*, and *Amaranthus spinosus*, accounting for the highest Summed Dominance Ratio (SDR), together contributing more than 50% of the total SDR. The remaining species individually contributed relatively small proportions to the total community dominance, indicating a community structure dominated by a few abundant species and many low-abundance species.

Based on SDR values, *P. repens*, *C. dactylon*, and *A. spinosus* were identified as the dominant weed species before herbicide application (Table 3). These dominant species persisted under shaded coffee canopy conditions, as similarly reported in other perennial cropping systems (Nguyen and Liebman 2022). The observed dominance occurred under moderately acidic soil conditions typical of highland coffee agroecosystems (Young and Hamerlynck 2023). At the initial observation stage, the dominance pattern indicates that the weed community was strongly structured by a few perennial species prior to herbicide application. Management implications related to long-term weed control are discussed in subsequent sections based on post-application observations (Waheed et al. 2022; Young and Hamerlynck 2023).

Several minor weed species, including *Galinsoga quadriradiata* and *Plantago major*, were present at low SDR values, indicating their limited contribution to overall community dominance at the initial stage (Kleiman and Koptur 2023). These low-abundance species formed part of the background weed flora prior to treatment, as commonly observed in coffee agroecosystems. Their presence contributes to overall species richness, but did not substantially affect dominance patterns at the pre-application stage (Clements and Jones 2021). Subsequent changes in the abundance of both dominant and minor species following herbicide application are examined in the following sections.

Weed dominance dynamics and diversity after glyphosate application

The dynamics of weed dominance and diversity, after glyphosate application, showed measurable changes over time based on numerical diversity and dominance indices. Glyphosate application was associated with a temporary reduction in weed diversity, while overall dominance structure remained relatively stable across observation periods. The present analysis focuses on changes in diversity indices, dominance values, and community similarity derived from field observations. These indices were calculated using pooled vegetation data across all treatments at each observation time and therefore represent site-level temporal patterns rather than treatment-specific responses.

Table 3. Composition of weed vegetation in the arabica coffee agroecosystem before herbicide application

Species name	Number of individuals	KR (%)	FR (%)	DR (%)	SDR (%)
Panicum repens L.	97	36.20	21.00	36.86	23.24
Cynodon dactylon (L.) Pers.	83	17.97	11.87	14.35	14.73
Galinsonga quadriradiata Ruiz & Pav.	56	12.12	11.87	8.06	10.68
Amaranthus spinosus L.	55	11.90	11.87	14.41	12.73
Plantago major L.	47	10.17	11.87	8.83	10.29
Paspalum conjugatum P.J.Bergius	36	4.77	7.79	4.85	7.61
Calyptocarpus sp.	22	4.76	5.09	3.61	4.49
Echinochloa colona (L.) Link	17	3.68	3.39	1.13	2.73
Portulaca oleracea L.	16	3.46	5.09	2.10	3.55
Marsilea sp.	7	1.52	1.70	0.90	1.37
Mitracarpus hirtus (L.) DC.	7	1.52	1.69	0.75	1.32
Drymaria cordata (L.) Willd. ex Schult.	5	1.08	1.70	0.90	1.23
Cyperus rotundus L.	5	1.08	1.69	0.83	1.20
Ageratum conyzoides L.	3	0.65	1.69	0.97	1.10
Sida rhombifolia L.	2	0.43	1.69	0.28	0.80
Crassocephalum sp.	1	0.22	1.69	0.06	0.65
Synedrella nodiflora (L.) Gaertn.	1	0.22	1.69	0.82	0.91
Bidens pilosa L.	1	0.22	1.69	0.26	0.72
Ceratopteris sp.	1	0.22	1.69	0.02	0.64
Total	462	100	100	100	100

Note: KR: Relative density, FR: Relative frequency, DR: relative dominance, SDR: Summed Dominance Ratio

In Tables 4 and 5, a consistent pattern is present: the diversity index (H´) declined from 2.27 to 2.12 on 14 days after application (DAA), then re-emerged between 28-42 DAA at 2.29-2.33. Dominance values (D) remained relatively stable, and community similarity to initial conditions was still high (>97%). The decline in diversity at 14 DAA coincided temporally with the period shortly after herbicide application, indicating a short-term reduction in community evenness (van Bruggen et al. 2021; Lisek 2023). At 28-42 DAA, the diversity index increased to values comparable with the initial condition, indicating a numerical recovery of species richness and evenness. This recovery occurred while community similarity values remained consistently high, suggesting that the same species assemblage persisted over time with shifting relative abundances without implying causal treatment effects. The observed patterns describe temporal changes in weed diversity and dominance without implying causal mechanisms related to organic amendments (Macias-Benitez et al. 2020; Obermeier et al. 2020). These results indicate that glyphosate application was associated with short-term changes in diversity indices rather than long-term shifts in dominance hierarchy, as observed at the site level (Pavlović et al. 2022).

Across all observation periods, community similarity values remained above 97%, indicating that species composition was largely conserved following glyphosate application. This result shows that observed changes primarily reflected shifts in relative abundance rather than species turnover. Several species with low SDR values at 14 DAA exhibited increased relative contributions at 28-42 DAA, contributing to the observed rise in the Shannon diversity index. This change reflects numerical variation in species proportions rather than confirmed ecological mechanisms (Pavlović et al. 2022; Lisek 2023). The recovery of these species explains the observed increase in the Shannon index during the later stages of observation.

Overall, the diversity and dominance indices indicate that glyphosate application was associated with a temporary reduction in weed diversity, followed by numerical recovery within 42 days after application (Ramula et al. 2022). Because no direct measurements of regeneration mechanisms were conducted, interpretations are limited to observed numerical patterns in diversity, dominance, and community similarity (Pavlović et al. 2022). Overall, weed diversity, dominance, and spatial distribution patterns in this study were analyzed descriptively at the site level and were not intended to test treatment effects of organic amendments or glyphosate doses. Therefore, herbicide-based suppression should be complemented with mechanical or ecological practices to manage persistent dominant species more effectively.

Spatial weed distribution pattern and environmental factors

The spatial distribution pattern of weeds describes how individuals of each species were distributed across sampling plots during the observation period. In this study, spatial patterns were evaluated using the SD/mean ratio to classify distributions as regular, random, or clumped. This approach represents simple dispersion index and does not incorporate spatial coordinates or spatial autocorrelation analysis, and the classification thresholds are sensitive to sample size. Therefore, these spatial patterns are descriptive, not statistically derived.

Table 4. Summed Dominance Ratio (SDR, %) weed on 0, 14, 28, and 42 DAA

Species name	SDR 0 DAA	SDR 14 DAA	SDR 28 DAA	SDR 42 DAA
Panicum repens L.	22.86	22.16	23.87	24.07
Cynodon dactylon (L.) Pers.	18.67	19.46	20.60	21.01
Amaranthus spinosus L.	14.29	14.37	14.57	14.44
Galinsoga quadriradiata Ruiz & Pav.	11.43	12.87	10.30	9.63
Plantago major L.	8.57	11.38	7.54	7.66
Paspalum conjugatum P.J.Bergius	6.10	8.68	6.28	6.13
Calyptocarpus sp.	4.19	3.89	4.77	4.81
Portulaca oleracea L.	3.43	2.40	3.02	3.06
Marsilea sp.	2.29	0.90	2.01	1.97
Drymaria cordata (L.) Willd. ex Schult.	1.90	0.60	1.51	1.53
Sida rhombifolia L.	1.33	0.30	1.01	1.09
Mitracarpus hirtus (L.) DC.	1.14	1.20	1.01	1.09
Cyperus rotundus L.	0.95	0.30	0.75	0.88
Ageratum conyzoides L.	0.76	0.30	0.75	0.66
Bidens pilosa L.	0.57	0.30	0.50	0.66
Ceratopteris sp.	0.57	0.30	0.50	0.44
Crassocephalum sp.	0.38	0.00	0.25	0.22
Eclipta prostrata (L.) L.	0.38	0.30	0.50	0.44
Axonopus compressus (Sw.) P.Beauv.	0.19	0.30	0.25	0.22
Total	100	100	100	100

Note: SDR (Summed Dominance Ratio) was calculated based on relative density and relative frequency of each weed species, DAA: days after herbicide application

Table 6 presents that dominant weed species such as *P. repens* and *C. dactylon* consistently exhibited regular distribution patterns across all observation times, while several minor species showed random or clumped distributions. These patterns indicate descriptively stable spatial arrangements of dominant species throughout the observation period.

The distribution categories for most species remained unchanged from 0 to 42 DAA, despite reductions in total individual numbers following glyphosate application. This result suggests that glyphosate application was associated with changes in weed density rather than shifts in spatial distribution categories (do Lago Lopes et al. 2020; Abalori et al. 2022; Lebbink and Fensham 2023). Because spatial distribution was assessed descriptively, conclusions are limited to observed patterns without inference on underlying ecological mechanisms (do Lago Lopes et al. 2020; Barbosa et al. 2024). From a management perspective, these observations suggest that preliminary, descriptive information on spatial occurrence may be relevant for improving control efficiency (Dobbs et al. 2022). Several minor species, including *A. conyzoides* and *Bidens pilosa*, consistently exhibited clumped distribution patterns across observation times. These patterns reflect localized aggregation of individuals rather than uniform spatial spread (do Lago Lopes et al. 2020; Barbosa et al. 2024).

Overall, dominant weed species tended to maintain regular spatial distributions, whereas less abundant species showed higher spatial variability. These observed patterns may provide an initial descriptive basis for considering spatially targeted weed management strategies based on distribution characteristics, potentially enhancing control effectiveness while reducing reliance on uniform herbicide application (Kumar et al. 2023).

Weed biomass response to glyphosate dose under different soil amendments at 28 DAA

Weed biomass responses to glyphosate application under different soil amendment treatments were evaluated based on dry biomass measurements collected at multiple observation times (0, 14, 28, and 42 DAA). Weed dry biomass data from all observation times were subjected to statistical analysis; however, a significant interaction between glyphosate dose and soil amendment was detected only at 28 DAA. Therefore, the presentation in Table 7 and Figure 1 focuses exclusively on the interaction effects observed at 28 DAA, while biomass responses at other observation times are described narratively. Analysis of variance (ANOVA) confirmed that glyphosate dose significantly affected weed dry biomass ($p<0.05$) at all observation times. The interaction between glyphosate dose and soil amendment was statistically significant only at 28 DAA, indicating a time-dependent interaction effect.

Table 5. Diversity index (H′), evenness (E), dominance (D), and community similarity (C′) were measured on 0, 14, 28, and 42 DAA

Observation	H′	E	D	C′
0 DAA	2.27	0.77	0.136	-
14 DAA	2.12	0.73	0.147	97.3 (vs 0 DDA)
28 DAA	2.29	0.83	0.148	97.4 (vs 0 DDA)
42 DAA	2.33	0.79	0.148	97.4 (vs 0 DDA)

Note: H': Shannon, E: Evenness, D: Dominance, C': Community similarity

Table 6. Weed distribution patterns based on total individual values (Σ), the standard deviation to mean ratio (SD/average), and distribution pattern categories at various observation times (0, 14, 28, and 42 DAA) in coffee fields

Weed name	Σ (0)	SD/Avg (0)	Spread (0)	Σ (14)	SD/Avg (14)	Spread (14)	Σ (28)	SD/Avg (28)	Spread (28)	Σ (42)	SD/Avg (42)	Spread (42)
Panicum repens	120	0.12	Regular	74	0.05	Regular	81	0.08	Regular	85	0.07	Regular
Cynodon dactylon	98	0.13	Regular	63	0.06	Regular	70	0.09	Regular	72	0.08	Regular
Amaranthus spinosus	75	0.10	Regular	55	0.07	Regular	60	0.10	Regular	62	0.09	Regular
Galinsoga quadriradiata	60	0.10	Regular	40	0.09	Regular	42	0.11	Regular	43	0.10	Regular
Plantago major	45	0.16	Regular	30	0.12	Regular	33	0.14	Regular	34	0.13	Regular
Paspalum conjugatum	32	0.17	Regular	24	0.15	Regular	26	0.16	Regular	27	0.15	Regular
Calyptocarpus sp.	22	0.26	Random	14	0.24	Random	15	0.25	Random	16	0.23	Random
Portulaca oleracea	18	0.27	Random	12	0.25	Random	13	0.26	Random	13	0.25	Random
Marsilea sp.	12	0.35	Random	8	0.33	Random	9	0.34	Random	9	0.33	Random
Drymaria cordata	10	0.43	Random	7	0.40	Random	8	0.41	Random	8	0.40	Random
Sida rhombifolia	7	0.50	Random	5	0.48	Random	5	0.49	Random	6	0.48	Random
Mitracarpus hirtus	6	0.56	Clumped	4	0.54	Clumped	4	0.55	Clumped	5	0.54	Clumped
Cyperus rotundus	5	0.71	Clumped	3	0.70	Clumped	3	0.71	Clumped	4	0.70	Clumped
Ageratum conyzoides	4	0.83	Clumped	3	0.82	Clumped	3	0.83	Clumped	3	0.82	Clumped
Bidens pilosa	3	1.25	Clumped	2	1.20	Clumped	2	1.22	Clumped	2	1.21	Clumped
Ceratopteris sp.	3	1.25	Clumped	2	1.20	Clumped	2	1.22	Clumped	2	1.21	Clumped
Crassocephalum sp.	2	1.67	Clumped	2	1.60	Clumped	2	1.63	Clumped	2	1.62	Clumped
Eclipta prostrata	2	1.67	Clumped	2	1.60	Clumped	2	1.63	Clumped	2	1.62	Clumped
Axonopus compressus	1	2.80	Clumped	1	2.70	Clumped	1	2.75	Clumped	1	2.72	Clumped

Note: Spatial distribution patterns were classified using the SD/mean ratio as a simple descriptive dispersion index, where values <1 indicate regular distribution, values close to 1 indicate random distribution, and values >1 indicate clumped distribution. This classification is descriptive and does not represent formal spatial statistical analysis

Table 7. Interaction effects of biochar amendment and glyphosate dose on weed dry biomass (g per 0.25 m², mean ± SD) at 28 days after application (DAA)

Glyphosate 486 g/l dose	Soil amendment		
	No amendment (b_0)	**Goat manure (b_1)**	**Biochar (b_2)**
g0: No herbicide	33.98±7.18^b	34.97±7.13^b	38.97±7.18^b
g1: 1,50 L. ha^{-1}	4.19±0.55^a	3.83±0.26^a	0.61±0.16^a
g2: 2,25 L. ha^{-1}	3.75±1.13^a	3.90±0.72^a	0.64±0.56^a
g3: 3,00 L. ha^{-1}	2.72±0.21^a	4.13±0.75^a	0.63±0.55^b
g4: 3,75 L. ha^{-1}	3.11±0.37^a	3.94±0.61^a	0.56±0.45^a
g5: 4,50 L. ha^{-1}	1.95±0.52^a	0.43±0.09^a	0.62±0.12^a

Note: Different letters within each column indicate significant differences among glyphosate doses within the same soil amendment (LSD, $p<0.05$). A significant interaction between glyphosate dose and soil amendment was detected at 28 days after application (ANOVA, $p<0.05$)

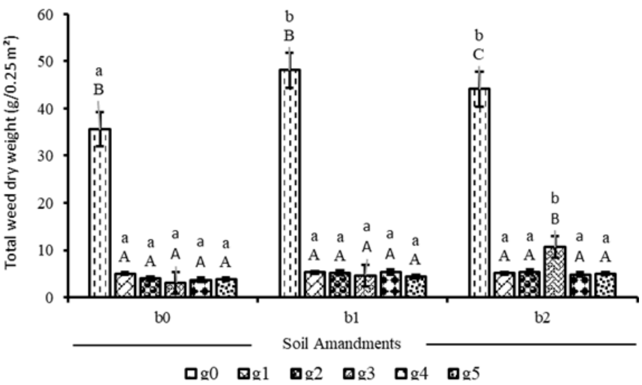

Figure 1. Weed dry biomass response to glyphosate dose within different soil amendments at 28 DAA

At 28 DAA, significant interaction effects between glyphosate dose and soil amendment were observed, with biochar-amended plots showing higher weed biomass at comparable glyphosate doses. Narrative observations from earlier and later sampling times (14 and 42 DAA) suggested temporal variation in weed biomass response; however, these trends are not shown in tabular form because no significant interaction effects were detected at those times. Because adsorption and degradation processes were not directly quantified, interpretation is limited to observed biomass responses rather than underlying mechanisms. Similar temporal patterns of reduced herbicide persistence under organic amendments have been reported previously (Mielke et al. 2022; Holatko et al. 2022).

The observed recovery of weed biomass suggests that organic amendments influenced the duration of effective weed suppression, although specific physicochemical and biological mechanisms were beyond the scope of this study (Holatko et al. 2022; Sharma 2025). Previous studies have reported that organic amendments can modify soil biological activity and herbicide persistence (Mielke et al. 2022). Interactions between herbicides and soil amendments have been reported to influence weed control effectiveness in other cropping systems (Polli et al. 2022). This agrees with the findings of (Takeshita et al. 2022), who reported that biochar alters herbicide degradation through sorption and changes in bioavailability, which depend on biochar's physicochemical properties and soil characteristics. Such interactions can simultaneously decrease herbicide efficiency while enhancing soil detoxification potential. From a cultivation perspective, this presents a dilemma: while biochar improves soil health, it simultaneously reduces glyphosate effectiveness (Sun et al. 2025).

From a weed management perspective, these results indicate that organic amendments may alter the temporal pattern of weed biomass response without overriding the dominant effect of glyphosate dose. Enhanced microbial respiration and enzymatic activity, as reported in previous studies (Yan et al. 2022), may accelerate herbicide degradation, thereby reducing persistence. Therefore, weed biomass recovery observed after 28 DAA should be interpreted as a temporal treatment response rather than as herbicide failure.

Practically, the selection of soil amendments should consider potential trade-offs between soil quality improvement and the duration of herbicide-based weed suppression. While biochar is widely recognized for improving soil properties, its application may be associated with shorter weed suppression duration under glyphosate-based control (Aon et al. 2023; Ramezanzadeh et al. 2023). In contrast, manure tended to maintain lower weed biomass for a longer period while supporting nutrient availability, although its soil-improving effects were less pronounced than biochar. Therefore, soil amendment selection should be aligned with weed management objectives and the desired balance between soil improvement and weed control duration (Zhao et al. 2022). Thus, references to temporal biomass recovery are based on descriptive interpretation of statistically analyzed data, not on values presented in Table 7 or Figure 1.

Herbicide persistence and microbial respiration in soil

The relationship between glyphosate persistence and microbial respiration is an important consideration in understanding the temporal dynamics of weed suppression. Glyphosate is a herbicide that can affect soil microbial communities (Ramula et al. 2022). Glyphosate persistence in the environment can lead to microbial community composition shifts, altering microbial respiration and the whole soil ecosystem (Tyler 2022). Such changes have been reported to coincide with shifts in soil biological activity, which may affect nutrient cycling and plant-microbe interactions (van Bruggen et al. 2021).

Figure 2 presents glyphosate residue dissipation (mg kg^{-1}) and microbial respiration (mg CO_2-C kg^{-1} day^{-1}) in soils amended with biochar, manure, or without amendment from 0 to 42 days after application (DAA). The figure illustrates inverse temporal trends between glyphosate residue decline and microbial respiration, which are presented descriptively without implying causality.

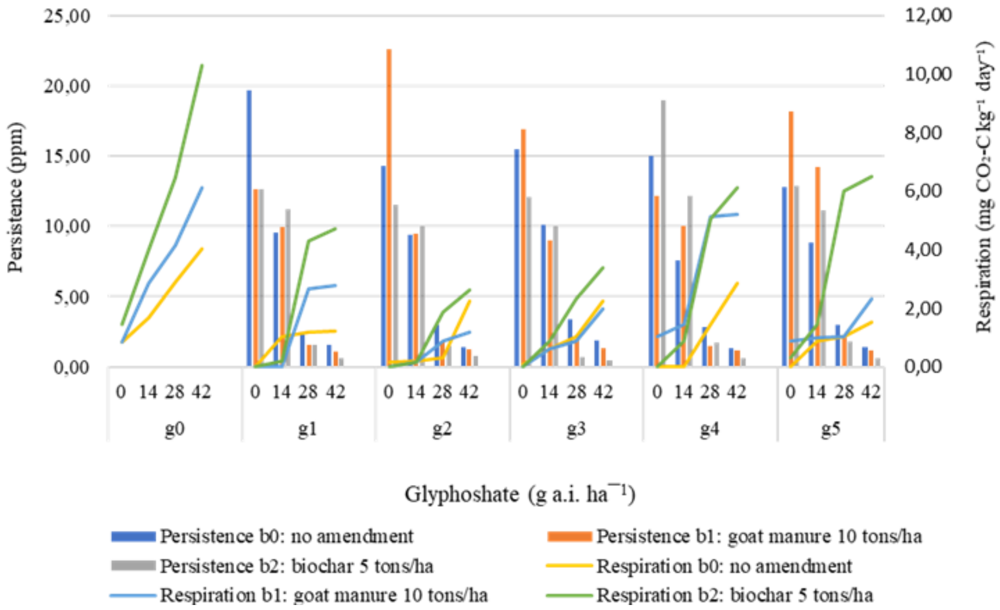

Figure 2. Glyphosate residue dissipation (mg kg^{-1}) and soil microbial respiration (mg CO - C kg^{-1} day^{-1}) in soils with biochar, manure, or no amendment from 0 to 42 days after application (DAA). Data are based on non-replicated composite samples and are presented descriptively

Figure 2 depicts glyphosate residue that declines at 14-28 DDA, showing a downward trend, with the pattern of microbial respiration that increased at the same period, although these trends should be interpreted cautiously because residue and respiration data were derived from non-replicated composite samples and are therefore indicative rather than statistically inferential. Increased respiration in biochar and manure may reflect elevated microbial activity (Omidvar et al. 2023), although specific microbial taxa and functional pathways were not measured in this study (Sibalekile et al. 2025). The biochar adsorption has been reported to reduce the bioavailable fraction of glyphosate (Klátyik et al. 2025), which may partly explain the lower residue levels observed; however, adsorption processes were not directly measured in this study. These processes are discussed here as hypothesized mechanisms may partly explain the observed residue decline, although their relative contributions cannot be confirmed from this dataset.

The concurrent decrease in residues and increase in respiration suggests a possible association, but no causal inference can be made. Since residue data were not replicated, these observations should be interpreted as indicative rather than conclusive. The biochar treatment showed the strongest indicative trend of residue decline. Such inverse trends have been reported in biologically active soils, but further replicated measurements would be needed to confirm this. Microbial stimulation may coincide with herbicide dissipation, but the timing and mechanisms remain uncertain. These patterns tentatively suggest that chemical and biological responses may occur in parallel.

The inverse relationship between glyphosate concentration and CO_2 evolution may indicate a concurrent pattern between microbial activity and residue decline. As residues decline, microbial utilization pathways reported in other studies were not assessed in this study (Klátyik et al. 2025; Sibalekile et al. 2025). The observed patterns may reflect concurrent changes, although the study design limits mechanistic interpretation. However, this study did not directly measure the adsorption of glyphosate. Because the residual data were not replicated, these results should be understood as an initial overview, not as a definitive conclusion.

The residue decline pattern in the biochar treatment aligns with previous reports describing biochar-glyphosate interactions, thereby reducing its toxicity to microorganisms. Nevertheless, this absorption process was not measured directly, so its explanation can only be inferred from the patterns observed in the study. Recent studies also show strong biochar-glyphosate interactions (Le et al. 2021; Rallet et al. 2022; Zuo et al. 2025), thus, multiple processes may contribute to the trends observed here.

The comparison between biochar and manure treatments showed variations in microbial response, with soil treated with biochar showing a greater indicative increase in CO_2 evolution (Aon et al. 2023; Ramezanzadeh et al. 2023). This suggests that amendment type may influence soil biological responses. Practically, faster residue decline may shorten the duration of effective weed suppression. This aligns with the observation that weed biomass increased again at 28-42 DAA as glyphosate residue levels declined, indicating a temporal association rather than a confirmed causal relationship. Persistence curves show that glyphosate concentrations decline rapidly within the first 14-28 days. This condition aligns with weed vegetation data, where diversity and dominance were observed to re-stabilize by 28 DAA. Biochar treatments showed lower residue levels, which may relate to adsorption or microbial interactions (Hasanan et al. 2024). Therefore, biochar was associated with lower residue levels and concurrent weed community responses (Mielke et al. 2022). Biochar treatments also increase microbial respiration, indicating that the soil is more biologically active (Yan et al. 2022). The elevated respiration coincided with periods of residue decline; the herbicidal effect may diminish more quickly in soils with higher biochar dosage (Tan et al. 2022).

This condition is crucial to consider in weed management, because although biochar has positive impacts on soil fertility, microbial ecosystem health, and herbicide dissipation, its application may also hasten the reduction of glyphosate effectiveness. The dynamics of weed communities in coffee plantations indicate a strong relationship between weed diversity, dominance, biomass, and environmental variables, including glyphosate persistence and soil microbial respiration (Maclaren et al. 2020). The decline in weed diversity index and biomass at 14 days after application (DAA) reflects the initial effectiveness of glyphosate, although weed community recovery resumed at 28-42 DAA as herbicide residues diminished.

Biochar improves soil biological conditions but may shorten herbicide persistence (Bagrecha et al. 2024; Chopra et al. 2024), which may be associated with faster glyphosate dissipation, thereby shortening the duration of weed control (Zhang et al. 2025). Weed resurgence reflects the interaction between herbicide dynamics and soil ecological conditions. Integrated management remains essential to maintain control effectiveness. (Messelhäuser et al. 2022), combining appropriately dosed herbicide application, biochar use, and mechanical and biological methods to ensure effective weed control without compromising sustainable agroecosystem (Wong et al. 2022; Nagargade et al. 2024).

The relationship between glyphosate residue reduction and increased microbial respiration suggests a possible association between chemical dissipation and biological activity. Elevated microbial activity under biochar treatment may reflect microbial responses influenced by biochar additions (Zhao et al. 2022). To maintain optimal weed control effectiveness, biochar dosage must be carefully regulated so that glyphosate remains available during the critical weed growth phase (Mustafa et al. 2022). Simultaneously, glyphosate dosage should be adjusted to provide sufficient suppression while avoiding unnecessary losses (van Bruggen et al. 2021). From an agroecological perspective, these findings reinforce the importance of integrating chemical weed control with non-chemical methods for ensuring both effectiveness and long-term sustainability (Mosa et al. 2023). Overall, the general sequence of weed suppression, residue decline, and community recovery observed in this study appears to occur over 42 days, although the underlying mechanisms cannot be fully confirmed due to the non-replicated nature of the residue data.

This integrative perspective reinforces the concept of ecological weed management (Pavlović et al. 2022), emphasizing the synergy between selective herbicide application, organic inputs, and biological control as complementary rather than opposing strategies. Such an approach can preserve belowground microbial networks while reducing glyphosate dependence, thereby enhancing the resilience of coffee agroecosystems in tropical environments. These findings highlight the potential role of organic amendments in mitigating herbicide residues and maintaining soil biodiversity, offering a practical approach for sustainable weed management in Arabica coffee systems.

Limitations of this study include the use of non-replicated composite samples for glyphosate residues, microbial respiration, and microbial identification, restricting these results to descriptive interpretation. In addition, microbial communities were characterized morphologically, without molecular confirmation, and mechanistic processes such as adsorption or biodegradation were not directly measured. Future studies should incorporate replicated residue and respiration measurements, molecular characterization of microbial communities, and direct assessment of sorption and degradation processes to clarify mechanisms underlying amendment-glyphosate interactions and to refine integrated weed management strategies in coffee agroecosystems.

In conclusion, the application of biochar and manure in Arabica coffee soils showed descriptive differences in weed biomass, microbial respiration patterns, and glyphosate dissipation under field conditions. This study demonstrates that glyphosate application in Arabica coffee agroecosystems was associated with short-term changes in weed communities and biomass, with outcomes influenced by organic soil amendments. A total of 19 weed species were recorded before treatment, dominated by *P. repens, C. dactylon,* and *A. spinosus*. Weed diversity (Shannon index) declined from H′ = 2.27 before application to 2.12 at 14 DAA, then recovered to 2.29-2.33 by 28-42 DAA, while community similarity remained high (>97%), indicating shifts in relative abundance rather than species turnover. Weed dry biomass was significantly affected by glyphosate dose at all observation times, and a significant glyphosate × soil amendment interaction occurred at 28 DAA, where biochar-amended plots showed higher weed biomass (~0.56-0.64 g per 0.25 m^2) than unamended plots at comparable doses, suggesting reduced persistence of effective weed suppression. Glyphosate residues showed a faster apparent decline in amended soils, particularly biochar-treated plots, over 14-42 DAA, concurrent with higher measured soil microbial respiration during the same period. These parallel trends suggest an association between amendment-driven soil biological activity and glyphosate dissipation, although no causal inference can be made.

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